EPIPHYTIC SEED MICROBIOMES OF WHEAT, CANOLA, AND LENTIL

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By

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ABSTRACT

Microorganisms are found colonizing all plant organs including seeds. Seeds are reproductive structures able to carry and transfer microorganisms from one plant generation to the next acting as an initial source of microbial inocula for the next generation of plants. Seed-associated microbial communities offer the potential of improving crop production and yield through protection against abiotic and biotic stresses. Despite their agricultural relevance, seed-borne bacterial and fungal communities as well as factors influencing their assemblage remain largely unknown. Therefore, the purpose of this study was to: *i*) characterize the seed-associated microbiomes of three agricultural crops important for food security; wheat, canola, and lentil, *ii*) explore genetic and environmental factors influencing the assembly of the microbiota carried by seeds, and *iii*) examine the preservation and transmission of seed microbiomes. To achieve these objectives seed samples of different lines harvested from different field locations, years, and generations were subjected to high-throughput amplicon sequencing of the bacterial 16S rRNA and the fungal internal transcribed spacer (ITS) regions. My results suggest recruitment, transmission, and preservation of seed-associated microbiota are determined mainly by the environment in which the plants are grown and to some extent by the host. In addition, a shared set of microorganisms (i.e., core microbiome) was found when seed microbiomes of different crops, lines, and from different sources (i.e., produced in different fields and years) were analyzed together. The existence of this core microbiome implies that plants recruit and carry bacterial and fungal species that could interact with further generations, affecting their adaptation and establishment in novel environments. Some members of this core including *Sphingomonas* sp., *Pantoea agglomerans*, and *Vishniacozyma victoriae* are reported to be beneficial to their hosts. In my study I also found evidence suggesting seed-associated microbial communities are vertically transmitted from the mother plant to the offspring. *Cutibacterium, Methylobacterium, Sphingomonas, Streptococcus*, and *Tepidimonas* were found across multiple generations of lentil seeds irrespective of the soil in which they were grown. These findings represent an important step toward the advancement of sustainable breeding and agricultural strategies to utilize microbial communities carried by seeds for their potential contribution to plant health and productivity.

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LIST OF ABBREVIATIONS

16S rRNA	16S Ribosomal RNA
AAFC	Agriculture and Agri-Food Canada
ABA	Abscisic acid
ABS	*Amblyseius cucumeris System*
ACC	1-Aminocyclopropane-1-Carboxylate
AMF	Arbuscular Mycorrhizal Fungi
ANCOM	Analysis of Composition of Microbiomes
ASV	Amplicon Sequence Variant
BCA	Biological Control Agent
CDC	Crop Development Centre
CEC	Cation Exchange Capacity
CFU	Colony-Forming Unit
cpn60 UT	Chaperonin 60 Universal Target
DOPE-FISH	Double labeling of Oligonucleotide Probes for Fluorescence *In Situ* Hybridization
ECM	Ectomycorrhiza
EDTA	Ethylenediaminetetraacetic Acid
FISH	Fluorescence *In Situ* hybridization
G	Generation
IAA	Indole-3-Acetic Acid
ISA	Indicator Species Analysis
ITS	Internal Transcribed Spacer
IV	Indicator Value
MF	Melfort
NAM	Nested Association Mapping
NF	Nitrogen-Fixing
OC	Organic Carbon
OM	Organic matter
OTU	Operational Taxonomic Unit

PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational Analysis of Variance
PGPR	Plant Growth Promoting Rhizobacteria
PSO	Phosphate-Solubilizing Organism
qPCR	Quantitative Real-Time Polymerase Chain Reaction
R	Replicate
RFO	Raffinose Family Oligosaccharide
S	Soil
SC	Scott
SCRDC	Swift Current Research and Development Centre
SEM	Scanning Electron Microscopy
SK	Saskatoon
sp.	Species
spp.	Species

1. INTRODUCTION

Feeding a growing world population while dealing with contemporary issues including climate change, land degradation, and cropland losses is a major challenge for food and nutritional security (Busby et al. 2017; Myers et al. 2017). Considering this scenario, the use of innovative farming strategies able to increase the productivity and sustainability of global agriculture is necessary to meet dietary needs and food preferences. Microbial communities such as bacteria and fungi play pivotal roles in a plant's adaptation, development, and response to biotic and abiotic stresses (Toju et al. 2018; Banerjee et al. 2018). Therefore, harnessing plant-associated microbial communities (i.e., the plant microbiome) is a promising alternative to increase crop yield while reducing the indiscriminate use of chemical-based fertilizers and pesticides that not only impact the environment but also human health (Vishwakarma et al. 2020; Tosi et al. 2020).

Extensive research demonstrates that microorganisms colonize all plant tissues and can increase plant growth, control pests and pathogens, and alleviate abiotic stresses including heavy metal contamination, soil salinity, and drought (Arif et al. 2020; Song et al. 2020). Few studies, however, have assessed the transmission and preservation of the plant's microbiome (Berg and Raaijmakers 2018; Wassermann et al. 2021). Seeds are reproductive structures involved in the transmission of plant microbiota from one generation to another, acting as the initial microbial inocula (Shade et al. 2017; Rezki et al. 2018). Consequently, investigating seed-associated microbial communities is crucial to better understand the assembly and inheritance of a plant's microbiome.

Saskatchewan is the largest producer and exporter of agricultural crops in Canada (Statistics Canada 2020a). Wheat (*Triticum aestivum*), canola (*Brassica napus*), and lentil (*Lens culinaris*) are among the major crops cultivated in this province and are considered staple crops around the world, essential to deal with malnutrition and food insecurity (Statistics Canada 2020a; Daryanto et al. 2017).

By improving our knowledge of seed microbiomes associated with these staple crops, we may be able to design and develop sustainable breeding and agricultural strategies for improving crop productivity, thus assuring food security in coming years. The overall objectives of this study were to (*i*) characterize the seed-associated microbiomes of wheat, canola, and lentil (*ii*) explore genetic and environmental factors influencing seed microbiome assemblage, and (*iii*) examine the preservation and transmission of seed microbiomes. To achieve these objectives, studies were designed to address the following hypotheses:

1) The host plant genotype (e.g., crop type, line) impacts the community structure of the seed microbiota.
2) Collective influences of the environment (e.g., location, harvesting year) have a stronger effect than plant genotype in shaping seed microbiomes.
3) Microbial communities are vertically transmitted from seed to seed across generations.

The following research thesis is presented in manuscript-style format. The thesis consists of a literature review (Chapter 2) followed by three research studies (Chapter 3, 4, and 5), a general discussion (Chapter 6), and future research directions (Chapter 7). Chapter 3 begins with the characterization of field-grown wheat, canola, and lentil seed microbiomes and an assessment of factors influencing seed microbiome assemblage. Chapter 4 provides an assessment of environment and plant genotype effects on the canola seed microbiome grown in different field environments. Chapter 5 examines vertical transmission of bacteria in lentil seeds across generations, as well as the impact of soil and genotype on the assembly of the lentil seed microbiome. Chapters 6, and 7 summarize research findings and provide ideas for future work. Each research study chapter is structured and written to stand alone for submission to peer-reviewed journals; however, each chapter includes a preface providing a transition from one study chapter to another. Due to the manuscript format, some redundant information may occur.

2. LITERATURE REVIEW

2.1. Food Security

According to the World Food Summit (1996), food security exists "when all people, at all times, have physical and economic access to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life". The demand for food is expected to rise by 70 to 100 percent by 2050 as a result of population growth (United States Department of Agriculture 2020a). Rapid population growth, climate change, soil erosion, water scarcity, and cropland expansion into unsuitable lands are some of the biggest challenges for food systems in the next few decades (Molotoks et al. 2021). Traditional agricultural practices will not be able to satisfy food needs, and thus the development and implementation of more sustainable food systems is needed to ensure food and nutrition security for billions of people around the world.

2.2. Agricultural crops

The global agricultural land area is 4.9 billion ha. Of this, one-third (1.6 billion ha) is currently used for cultivation of annual and perennial crops, with the other two-thirds for permanent meadows and pastures (wild prairie or grazing land used for five years or more) (Food and Agriculture Organization of the United Nations 2020). In Canada, about 37.8 million ha are dedicated to growing crops of which 31.2 million ha are located in the Canadian Prairies, with Saskatchewan the largest crop-producing province (16.4 million ha) (Statistics Canada 2020b).

Approximately 2,500 plant species have undergone some degree of domestication, and 250 species are considered to be fully domesticated for agricultural purposes; providing food, fiber, and biofuels for almost 8 billion people around the world (Moshelion and Altman 2015; Smýkal et al. 2018). Cereals, legumes, and roots/tubers represent the major food source for humans, which contribute essential nutrients to diets (Moshelion and Altman 2015; Daryanto et al. 2017). Major field crops in Canada include wheat, canola, maize, barley, soybean, and lentil, which are cultivated for domestic consumption and export. Currently, the largest three field crops in Saskatchewan are wheat, canola, and lentil with 5.2, 4.6, and 1.5 million ha seeded in 2020 (Statistics Canada 2020a).

2.2.1. Wheat

Wheat (*Triticum aestivum*) is grown more widely than any other crop in a variety of climates, ranging from hot and dry locations in Africa, Asia, and Australia to more favourable environments in Western Europe and North America (Gazal et al. 2017). Moderately tolerant to drought and soil salinity, this monocot develops better under low humidity conditions (Steduto et al. 2012). Canada is the sixth largest producer of wheat in the world after China, India, Russia, United States, and France (FAOSTAT 2020). This member of the Poaceae family is the major crop in Canada with 32 million tonnes harvested in 2018, of which over 45% were produced in Saskatchewan (Statistics Canada 2020a). Wheat is an important diet component, providing approximately 15% of calories consumed by humans daily in a variety of products including breads, biscuits, cakes, pasta, noodles, couscous, beer, and others (Stirling et al. 2014; Balfourier et al. 2019). Although most wheat is cultivated for human consumption, this cereal sometimes replaces corn and barley in livestock feed. Wheat is also utilized as a feedstock for generation of renewable fuels and in recent years has become the second most used starchy crop after corn in bioethanol manufacture (Mohanty and Swain 2019).

2.2.2. Canola

Canola (*Brassica napus* L.) is an oilseed rape developed in Canada in 1974 from backcrosses designed to transfer low erucic acid and low glucosinolate characteristics for improved nutritional value (Stefansson and Kondra 1975). Nevertheless, "canola" is a generic term used to describe *Brassica* species (*B. napus*, *B. rapa*, or *B. juncea*) varieties containing "less than 2% of erucic acid and less than 30 μmol of glucosinolates per gram of air-dried, oil-free meal" (Government of Canada 2017; Canola Council 2020a). This agricultural crop contributes more than $26 billion annually to the Canadian economy and the canola sector employs about 250,000 people (Agriculture and Agri-Food Canada 2019). Canada exports more than 90% of its canola to nearly 50 markets all over the world (Canola Council 2020b). Australia and the European Union also cultivate spring and winter canola to produce high quality edible oil and animal feed (McVetty et al. 2016). Canola oil contains low levels of saturated fatty acids (approximately 7%) and significant amounts of oleic acid (approximately 61%), linoleic acid (approximately 21%), and α-linolenic acid (approximately 21%), which have been associated with several cardioprotective effects (Lin et al. 2013). In addition, canola is a source of protein with high bioavailability and digestibility.

The main non-edible use for canola is biodiesel production; however, it is also used by cosmetic, coolant, and printing industries (McVetty et al. 2016).

2.2.3. Lentil

Lentil (*Lens culinaris*), a self-pollinating dicot and a cool season pulse species, is the oldest cultivated legume (Southwestern Asia, 7000 bc) (Nleya et al. 2004; Muehlbauer et al. 2006). This crop is grown annually in semi-arid regions around the world including North America, Asia, Australia, Ethiopia, and others. Annual global production of lentil ranks fifth (5 million tonnes) among legumes after dry bean (24 million tonnes), chickpea (13 million tonnes), dry pea (11 million tonnes), and cowpea (7 million tonnes). Lentil production in Canada began in 1969 and currently Canada is the largest producer in the world with 2.1 million tonnes harvested in 2018 (Food and Agriculture Organization of the United Nations 2019; Statistics Canada 2020a). In rotation with cereals, this pulse provides several agronomic benefits including biological nitrogen fixation, which replaces synthetic fertilizers, thereby reducing negative environmental impacts (Rodda et al. 2017). Another important characteristic of this pulse is its use to break disease and pest cycles in crop rotations (MacWilliam et al. 2014). In addition to having high fiber and protein content, lentils are rich in carbohydrates, vitamins, and minerals (Hefnawy 2011). These nutritional properties make this member of the Fabaceae family a crucial dietary component to deal with malnutrition. Lentil seeds are also used as source of starch for textile and printing industries (Singh and Singh 2014).

2.3. Sustainable crop production

The implementation of sustainable food production systems and resilient agriculture is crucial to meet the needs of a growing population (Fraser et al. 2016). Novel technologies based on yield improvement while reducing environmental impacts are gaining attention due to the vast advantages they provide over traditional intensive and extensive agricultural practices. For example, the improvement of photosynthetic efficiency through genetic engineering has the potential to increase crop productivity, while decreasing the use of water and nutrient inputs (Simkin et al. 2019). Another possible approach is harnessing plant-microbe associations, which represent one of the key determinants of plant health and productivity (Schlaeppi and Bulgarelli 2015; Mahadevakumar and Sridhar 2020).

2.4. The plant microbiome

Plant species form complex associations with a plethora of microorganisms that contribute significant genetic information influencing plant performance and survival (Rosenberg and Zilber-Rosenberg 2016; Cordovez et al. 2019; Hawkes et al. 2020). Bacteria, fungi, oomycetes, viruses, archaea, and protists colonizing external and/or internal plant tissues are considered the "plant microbiome" (Turner et al. 2013; Berg et al. 2014a; Levy et al. 2018; Trivedi et al. 2020). Microorganisms interacting with plants can exert beneficial, neutral, or detrimental effects including symbioses, mutualism, competition, predation, and pathogenesis (Fig 2.1). Nutrient uptake, plant growth promotion, stress tolerance, and pathogen resistance are among the beneficial functions microorganisms provide to their host (Quiza et al. 2015; Leach et al. 2017; Compant et al. 2019). The establishment and composition of these microbiomes depend on abiotic factors such as soil type, climatic conditions (e.g., rain, wind, temperature, UV radiation), and anthropogenic activities. Biotic factors also impact the plant microbiome. These include host genotype and interactions at multiple trophic levels that regulate microbial community structure. Nematodes, microarthropods, and saprophagous soil animals can potentially generate trophic cascades, thus influencing the overall microbiome assembly and dynamics (Thakur and Geisen 2019; Singh et al. 2020).

Recent advances in culture-independent high-throughput sequencing technologies significantly increase our understanding of plant microbiomes (Lundberg et al. 2013; Agler et al. 2016; Regalado et al. 2020). For instance, it is known that management practices such as crop rotation impact the rhizosphere microbiome, with larger effects observed in fungal than bacterial communities (Benitez et al. 2017; Ai et al. 2018; Maarastawi et al. 2018). Similarly, weather-related events such as daily precipitation can affect the dynamics and activity of the phyllosphere microbiota (Copeland et al. 2015; Allard et al. 2020). As more knowledge of plant microbiomes in natural and agricultural systems becomes available it will support sustainable farming practices by providing insights into critical plant-microbe interactions.

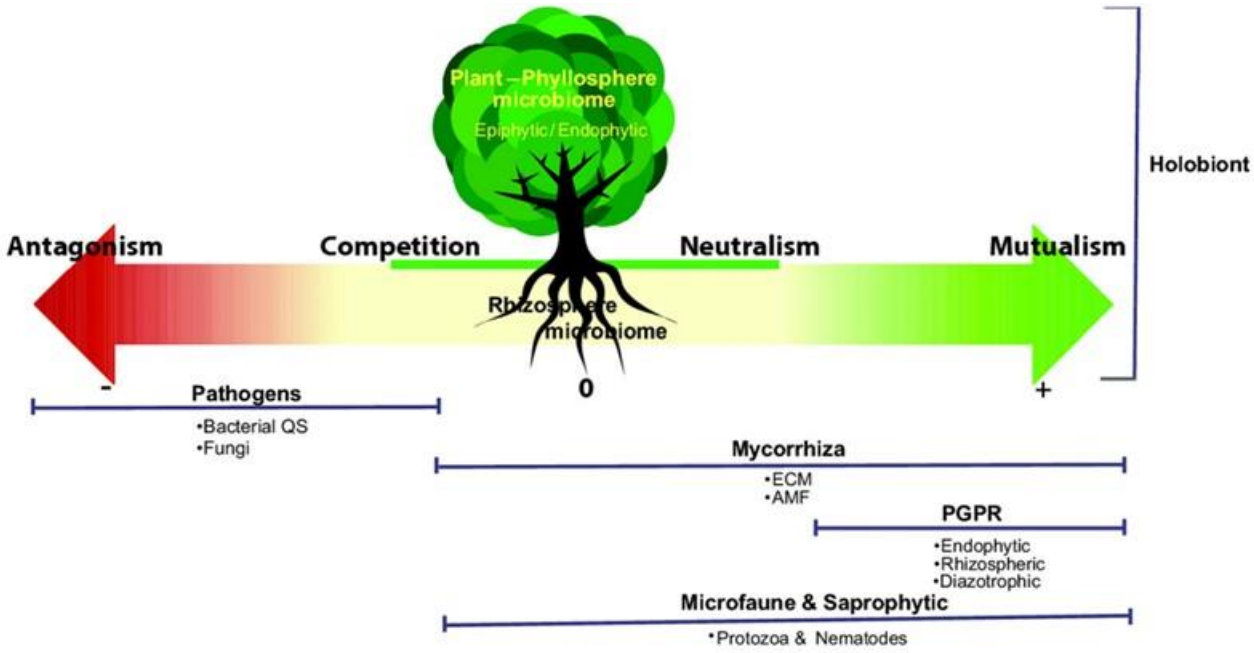

Figure 2.1 Examples of ecological interactions within the plant-microbiome. Many microorganisms participate in these interactions with host plants: ectomycorrhiza (ECM), arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR), phosphate-solubilizing organisms (PSOs), endophytes, epiphytes, and macrofauna (Quiza et al. 2015).

2.5. Seeds

Seeds are considered an ultimate product of land plant evolution. These sophisticated reproductive structures are responsible for the rapid evolutionary radiation and diversification of the spermatophyta division (Baroux and Grossniklaus 2019). A seed is an embryo encapsulated in a protective and nourishing structure that emerges only when appropriate conditions such as temperature and moisture are met (Fenner and Thompson 2005). There is immense diversity in the internal and external structure of seeds, but the three basic components are the embryo, endosperm (or perisperm), and seed-coat (Boesewinkel and Bouman 1984; Nelson 2004).

Seeds can survive long periods of time under extreme conditions, which facilitates the dispersal, adaptation, and persistence of plant species in a wide range of ecosystems (Nelson 2018). Most agricultural crops are grown from seeds, making them more valuable to humans than any other plant organ (Schwinn 1994). In most crops, seeds are considered the starting unit; however, they also may be the end point (Maude 1996). These vital organs contain carbohydrates, oils, and proteins to support early seedling development as well as the growth and proliferation of microbial communities associated with seeds. For example, wheat seeds contain approximately 71% carbohydrates, 13% protein, 11% water, and 2% total lipids (Shewry and Halford 2002). Canola seeds contain about 38-45% oil, 17-26% protein, and 20% carbohydrates. Lastly, lentil seeds are composed of 63% carbohydrates, 24% protein, 8% water, and 1% total lipids (Anwar 2015; United States Department of Agriculture 2020b). Other compounds including alkaloids, lectins, proteinase inhibitors, phytin, and raffinose family oligosaccharides (RFOs) are also present in seeds (Sliwinska and Bewley 2014).

2.6. The seed microbiome

The seed microbiome is defined as the microbial communities carried by seeds that show beneficial, neutral, or deleterious effects on plant fitness. These communities can be found on the seed surface or imbedded in internal tissues (Barret et al. 2015). First reports of seed-associated microorganisms date back to 1755 when Tillet described that wheat seeds carried a plant parasite responsible for bunt disease (*Tilletia* spp.). A century later, in 1892, Beach reported the first seed-borne bacteria, *Bacterium phaseoli* (*Xanthomonas axonopodis*) (Orton 1931; Srivastava et al. 2020). In 1898, fungal endophytes were discovered in grass *(Lolium temulentum* L.) seeds (Vogl 1898). Years later it was proven that these fungi belonged to the *Epichloë* genus (White et al. 1996).

By 1931, fifty-three microorganisms were reported as seed-borne in pulses, vegetables, forage crops, flax, flowers, and tubers (Alcock 1931).

To date, most of the information available regarding seed-associated microbiota was collected from cultured-based studies, with a major focus on endophytes. Nevertheless, innovations in microscopy and high-throughput sequencing methods allow the identification, characterization, and quantification of nonculturable microorganisms associated with seeds (Nelson 2018).

2.6.1. Origin of seed-associated microbial communities

In general, the plant microbiome can be acquired vertically from the mother plant (generation to generation) or horizontally from the surrounding environment (Johnston-Monje et al. 2016; Nelson et al. 2018). Several transmissions routes are described in the literature, including transmission via seed, colonization of the spermosphere (area around germinated seeds), colonization of developing reproductive organs (via xylem vessels or via the shoot apical meristem), colonization of root from soil, colonization of leaves though stomata via air (rain, wind), transmission via sap-feeders, and transmission to flowers via pollinators (Frank et al. 2017) (Fig 2.2).

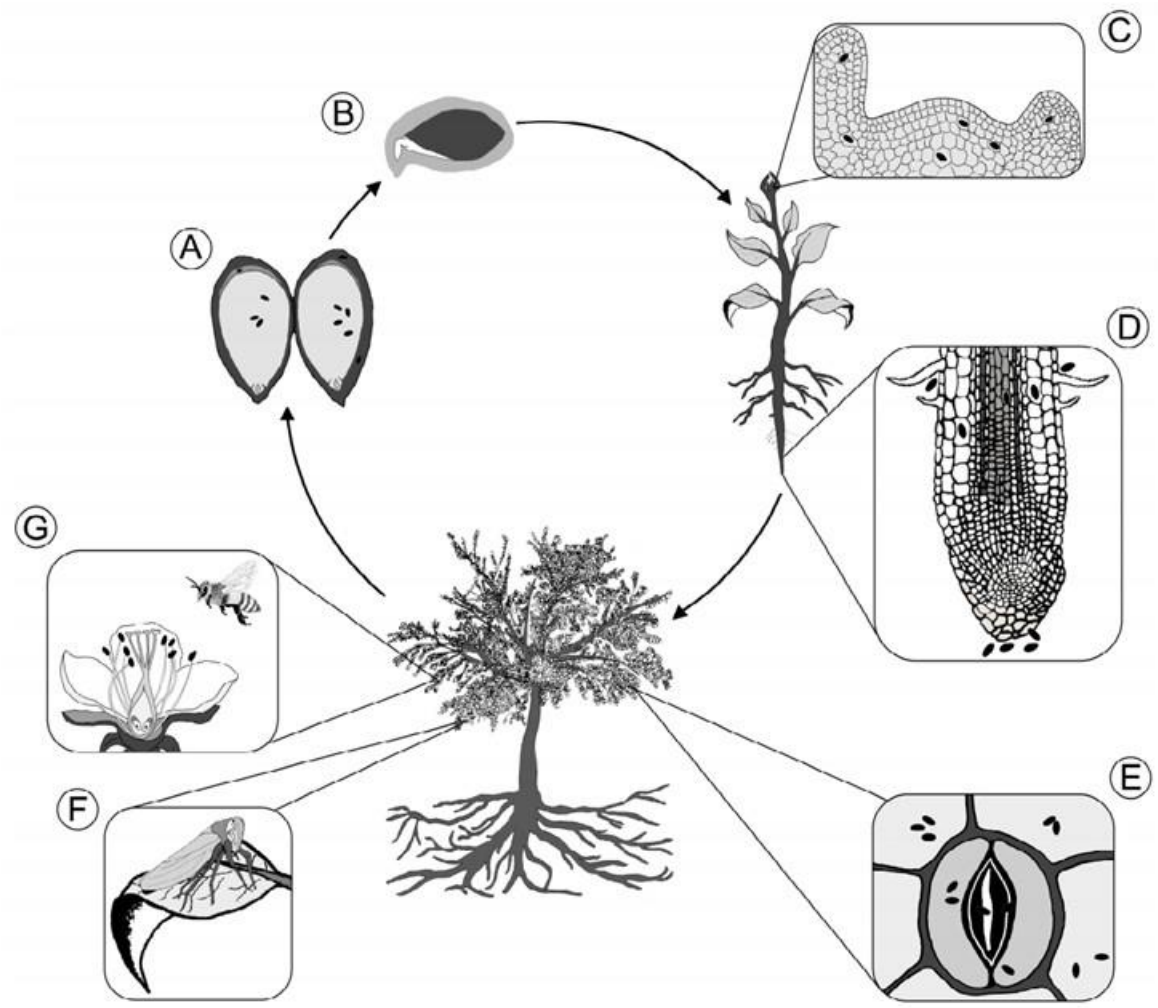

Figure 2.2 Transmission routes of microbial communities across the plant life cycle via seed **(A)**, colonization of the spermosphere **(B)**, colonization of developing reproductive organs **(C)**, colonization of root from soil **(D)**, colonization of leaves though stomata after transmission via air (rain, wind) **(E)**, transmission via sap-feeders **(F)**, transmission to flowers via pollinators **(G)** (Frank et al. 2017).

Although few studies have focused on the origin of seed-associated microbial communities, information collected from phytopathological studies show microbes can be vertically transmitted via vascular connections, gametes, or reproductive meristems. For instance, an indirect transmission via gametes occurs when pollen-associated microbes are transported to the stigmas of flowering plants, moved through the style and micropyles, thereby delivering the microbes along with the male gametes into the embryo sac. This route of transmission is mainly observed in biotrophic fungi including *Ustilagum segetum,* in which the teliospores reach the stigma, the hyphae grow between the cells of the stylar canal, penetrate the inner integument to the nucellus, to finally colonize the young endosperm and embryo (Gaumann 1950; Bashyal et al. 2020). Another indirect path of microbial transmission is via flowers or fruits, thus microorganisms colonizing petals, pistils, and the pericarp are transferred to seeds (Maude 1996; Truyens et al. 2015). In some cases, necrotrophic pathogens infect the fruit surface (i.e., pods), consequently infecting the seeds, as is the case of *Alternaria* spp., which penetrate seed coat tissues either directly through the cuticle or through the hilum (Vaughan et al. 1988; Gaur et al. 2020). Several phytopathogens can be transmitted via seeds, which means seeds act as a vector and facilitate pathogens dispersion in new regions and pathogens survival across growing seasons (Rennie 1998).

Microorganisms offering positive effects to the host also are vertically transmitted. For example, *Epichloë* spp. are fungal symbionts of cool-season grasses and responsible for conferring resistance to insect pests, and transmitted vertically by seeds to the next generation. These fungi are able to colonize the ovules and seed tissues during flowering and seed formation stages (W. Zhang et al. 2017; Gagic et al. 2018). Similarly, *Azospirillum brasilense,* a plant growth-promoting bacterium is vertically transmitted in beans (*Phaseolus vulgaris*), in which flowers seem to produce a chemoattractant that promotes *Azospirillum* seed colonization (Malinich and Bauer 2018).

The acquisition of seed-associated microorganisms is not limited to vertical transmission from the parent, as it can also occur through contact with microbial sources present in the ecosystem. Thus, air-borne, soil-borne, and others microorganisms can be horizontally transmitted to seeds (Shade et al. 2017; Nelson 2018). Consequently, microorganisms from the surrounding environment are able to colonize seed compartments during agricultural processes such as harvesting, threshing, and storage (Singh and Mathur 2004; Chen et al. 2016). Any of these transmission pathways (vertical or horizontal) may also influence the location where the microorganisms can be found within the seed tissues (Shade et al. 2017).

2.6.2. Microbial niches within the seeds

There is evidence that microbial communities can colonize both the epiphytic compartments including the seed coat and/or husk and the endophytic compartments including the endosperm and/or embryo. For example, Glassner et al. (2017) used scanning electron microscopy (SEM) and confocal laser-scanning microscopy with double labelled oligonucleotide probes for fluorescence *in situ* hybridization (DOPE-FISH) to show different colonization niches in seed tissues of melon (*Cucumis melo* L.). The main findings of their work showed that in *C. melo* L. Betaproteobacteria colonized only the outer seed coat while Alphaproteobacteria colonized the seed coat and the envelope surrounding the embryonic hypocotyl-root tissues. In contrast, Gammaproteobacteria and Firmicutes were mostly detected in the cotyledons. Similarly, Mitter et al. (2017) described bacterial cells colonizing the embryo of pepper (*Capsicum annuum*) and soybean (*Glycine max*) seeds using fluorescence *in situ* hybridization (FISH) targeting the 23S rRNA gene of a vertically transmitted strain of *Paraburkholderia phytofirmans*. Likewise, *Candidatus Burkholderia kirkii*, a vertically transmitted endophyte in dotted wild coffee (*Psychotria punctata*) was found inhabiting the shoot apical meristem of the seed embryo (Sinnesael et al. 2018).

2.6.3. Characteristics of seed-borne microorganisms

Conditions inside and outside the seed are not suitable for most microorganisms. Consequently, communities inhabiting the seeds tend to exhibit tolerance to the high osmotic pressure conditions due to starch accumulation and loss of water. The ability to use phytate as phosphate source, as well as formation of spores or cysts under limited nutrient conditions, are usually observed in microorganisms colonizing the seed habitat (Truyens et al. 2015; Cope-Selby et al. 2017). Plant growth promoting (PGP) traits including motility, nitrogen fixation, phosphate solubilization, siderophore production, antibiosis, ACC (1-aminocyclopropane-1-carboxylate) deaminase, cellulase, pectinase, and protease activity have also been observed in the seed microbiota (Johnston – Monje and Raizada, 2011; Khalaf and Raizada 2016; Shahzad et al. 2018).

2.6.4. Transmission of microorganisms from seed to the growing plant

Microorganisms naturally carried by seeds are found in different stages of the plant growth. Huang et al. (2016) showed that bacteria (*Erwinia* and *Rhizobiales*) and fungi (*Emericella*) initially harbored in *Triticum aestivum* seeds were also found in the sprouts, demonstrating the preservation of microbial communities during plant development. Barret et al. (2015) found similar results in

members of the Brassicaceae family while exploring microbial communities during seed germination and emergence, showing the seed is a source of microbial inocula for the seedling. A study carried out by Kuźniar et al. (2020a) revealed that members of the *Pseudomonas* genus accompanied wheat cultivars from the endosperm stage to the development of the leaf, suggesting these bacteria play an essential role in their host fitness. A survey on large perennial reed grasses (*Phragmites*) revealed that seed-borne *Pseudomonas* spp. become intracellular endophytes in seedlings of Bermuda grass (*Cynodon dactylon*) and annual bluegrass (*Poa annua*) by colonizing root meristems and entering meristematic cells during the germination process. These endophytes increased plant growth and inhibited the fungal pathogen *Sclerotinia Homeocarpa* (White et al. 2018). Similarly, Verma et al. (2017) showed that seed-associated bacteria *Enterobacter asburiae*, *Pantoea dispersa*, and *Pseudomonas putida* modulated seedling development in rice, and not only increased root and shoot lengths, but also protected the plant against *Fusarium oxysporum*. Collectively, these observations suggest seed microbiota could be potential biotechnological tools for different functions i.e., plant growth promotion, antagonism against pathogens, etc.

2.6.5. The seed microbiome as potential biotechnological tools

Several reports in the literature demonstrate seed-borne endophytes offer beneficial effects to the host plant. Plant growth promotion traits including phosphate solubilization, indole acetic acid biosynthesis (IAA), and siderophore production are found in bacterial isolates recovered from the seed endosphere of maize (*Zea mays*) (Johnston-Monje and Raizada 2011; Chowdhury et al. 2019), wheat (*Triticum aestivum*) (Díaz Herrera et al. 2016), rice (*Oryza sativa*) (Ruiza et al. 2011; Pal et al. 2019), barley (*Hordeum vulgare*) (Rahman et al. 2018), tomato (*Lycopersicum esculentum*) (Xu et al. 2014), alfalfa (*Medicago sativa*) (López et al. 2018), browntop millet (*Urochloa ramose*) (Verma and White 2018), and others (Verma and White 2019). Similarly, N_2 fixing bacteria are found in the seed interior of cereals (Zawoznik et al. 2014; Liu et al. 2017), legumes (Chimwamurombe et al. 2016), and gourds (Khalaf and Raizada 2016). Abscisic acid (ABA) production leading to increase plant resistance to salinity stress is another attribute found in endophytes recovered from seeds as seen in *Bacillus amyloliquefaciens* isolated from rice (Shahzad et al. 2017). Protection against pathogens provided by bacterial and fungal species inhabiting seed tissues is one of the most studied areas in seed microbiome studies. Members of the *Pantoea* genus recovered from rice and wheat seeds displayed antagonism against phytopathogens including *Curvularia* sp., *Fusarium oxysporum, Fusarium graminearum*, and

Pythium ultimum (Ruiza et al. 2011; Díaz Herrera et al. 2016). Meanwhile, *Bacillus* and *Paenibacillus* species retrieved from domesticated cucurbits seeds showed antagonism against fungal and oomycetes pathogens including *Rhizoctonia solani*, *Phytophthora capsica*, and *Pythium aphanidermatum* (Khalaf and Raizada 2018). Likewise, members of the *Absidia* and *Acremonium* genera isolated from rice seeds inhibited *Magnaporthe grisea* growth (Atugala and Deshappriya 2015; Etesami and Alikhani 2016). Interestingly, seed endophytes not only produce antibiotic substances to control plant pathogens, they can also induce systemic resistance with specific effects on plant defense pathways. For example, *Bacillus amyloliquefaciens* YN201732 isolated from tobacco (*Nicotiana tabacum*) seeds induced resistance to powdery mildew by regulating chitinase and polyphenol oxidase activity (Jiao et al. 2020).

Although most seed microbiome studies focus on endophytes, there is now renewed interest in seed epiphytes (Mano et al. 2006; Barret et al. 2015). Morella et al. (2019) reported that *Pantoea agglomerans* and *Pantoea dispersa* isolates recovered from the surface (spermoplane) of tomato seeds, protected the host against the bacterial pathogen *Pseudomonas syringae*. Similarly, Links et al. (2014) found *Pantoea* species in wheat seeds were able to suppress *Alternaria* spp. Nevertheless, seed epiphytes are recognized for more than acting as biological control agents. Gholamalizadeh et al. (2018) described members of the *Pantoea* genera colonizing rice seed surface able to enhance seed germination and plant growth.

The manipulation and exploration of microorganisms naturally carried by seeds is gaining more and more attention. High-throughput sequencing technologies are leading to a better understanding of the culturable and nonculturable seed-associated microbiota as well as factors influencing their assembly (Wasserman et al. 2019). One of the most common approaches to study seed microbiomes is by amplicon sequencing of bacterial and fungal genes (Table 2.1). Studies revealed host genotype impact microbial community composition and assemblage in seeds, as reported in *Brassica napus* (Rybakova et al. 2017), *Cucurbita pepo* (Adam et al. 2018), and *Oryza sativa* (Eyre et al. 2019; Kim et al. 2020). Similarly, seed microbiome studies showed bacterial and fungal communities are shared across different genotypes in *Nicotiana tabacum* (Chen et al. 2020) (Table 2.1). Moreover, the identification of seed-borne antagonists against plant pathogens has been possible through amplicon sequencing of bacterial genes in *Triticum* spp. and *Brassica* spp. seeds (Links et al. 2014).

Table 2.1 Agricultural seed microbiomes detected using high-throughput sequencing technologies.

Plant species	Study focus	Methodology	Summary of findings	Reference
Triticum spp. *Brassica* spp.	Bacterial and fungal epiphytes associated with six wheat and five canola lines	Pyrosequencing of the *cpn60* UT amplicons	-A bacterial and fungal core microbiome shared among all lines was identified in each plant genus -*Pantoea agglomerans* strains isolated from the seeds showed antagonism against the seed-borne pathogen *Alternaria* sp.	Links et al. 2014
Brassica napus	Bacterial endophytes associated with three winter cultivars	llumina MiSeq amplicon sequencing of the 16S rRNA gene	-The seed microbiome was cultivar-specific -One third of the OTUs found were shared between the cultivars -Cultivars with higher bacterial diversity exhibited higher colonization resistance against *Verticillium longisporum*	Rybakova et al. 2017
Cucurbita pepo	Bacterial communities associated with fourteen genotypes	llumina MiSeq amplicon sequencing of the 16S rRNA gene	-The genotype highly impacted microbial community composition	Adam et al. 2018
Oryza sativa	Bacterial and fungal communities associated with two genotypes harvested from two different years and locations. Additionally, microbial communities associated with four seed compartments (grain, outer grain, husk, and outer husk) were explored	llumina MiSeq amplicon sequencing of the 16S rRNA and ITS regions	-A bacterial (*Sphingomonas*, *Methylobacterium*, Enterobacteriaceae members) and fungal (*Alternaria*, *Hannaella*, Pleosporales members) core microbiome shared among all samples -The microbial communities did not differ between years, locations, or genotypes. However, they differed by seed compartment - More unique amplicon sequence variants were identified in the outer seed husk	Eyre et al. 2019
Nicotiana tabacum	Bacterial endophytes associated with four cultivars	llumina HiSeq amplicon sequencing of the 16S rRNA gene	-Bacterial community structure did not differ between cultivars -A core microbiome shared among all cultivars was identified -Cultivars from the same breeding line shared a higher number of OTUs	Chen et al. 2020
Oryza sativa	Bacterial and fungal endophytes associated with forty-tree accessions: eighteen wild and twenty-six domesticated	llumina MiSeq amplicon sequencing of the 16S rRNA and ITS regions	-Evidence that speciation and domestication shape seed bacterial and fungal community structures -Genotype influences microbial composition - Seed microbiota can be vertically inherited	Kim et al. 2020

There is now renewed interest in how plants acquire and transfer (i.e., inherit) seed microbiomes. Mitter et al. (2017) described a new approach to manipulate seed microbiomes by inoculating the flowers of parent plants. The endophyte and plant growth promoting bacterium *Paraburkholderia phytofirmans* PsJN was introduced into monocot (maize and wheat) and dicot (pepper and soybean) seeds by inducing a vertical inheritance to the offspring. After spraying flowers with bacterial solutions adjusted to 10^8 CFU.mL^{-1}, bacterial cells were recovered from seeds produced under greenhouse conditions. In addition, the vertical transmission of *P. phytofirmans* was corroborated in field experiments using wheat plants. Techniques such as fluorescence *in situ* hybridization (FISH), quantitative polymerase chain reaction (qPCR), and 16S rRNA gene sequencing confirmed PsJN transmission from the parents to the offspring. Mitter et al. (2017) also verified that plant growth promoting effects including a faster development and earlier spike emergence were retained in the offspring. Similarly, Vujanovic et al. (2019) demonstrated the transgenerational transmission of the fungal seed-endophyte *Penicillium* sp. SMCD 2318 after inoculation of this strain in wheat seeds. These studies confirmed that it is possible to induce the acquisition and inheritance of the seed-associate bacterial and fungal communities, which represents a potential strategy for improving the sustainability of agricultural systems.

Wheat, canola, and lentil are among the stable crops used to feed people all over the world, and thus crucial for food security. Plant-microbe interactions are important for crop resilience and productivity, and should be important considerations in breeding strategies. Thus, future research should focus on understanding how plant microbiomes (especially in seeds) are assembled, transferred, and preserved in agricultural crops.

3. CROP, GENOTYPE, AND FIELD ENVIRONMENTAL CONDITIONS SHAPE BACTERIAL AND FUNGAL SEED EPIPHYTIC MICROBIOMES[1]

3.1. Preface

Microbial communities colonizing plant tissues have the potential to highly influence plant performance, health, and competitiveness. Numerous studies have explored plant-associated microbial communities in belowground plant organs; however, aboveground microbial communities have been overlooked in the literature. In this chapter, bacterial and fungal communities associated with seeds of different agricultural crops (*Triticum aestivum*, *Brassica napus*, and *Lens culinaris*) were characterized to assess factors influencing microbial assemblage in these reproductive organs.

3.2. Abstract

Seeds are reproductive structures able to carry and transfer microorganisms that play an important role in plant fitness. Genetic and external factors are reported to be partly responsible for the plant microbiome assemblage, but their contribution in seeds is poorly understood. In this study, wheat, canola, and lentil seeds were analyzed to characterize diversity, structure, and persistence of seed-associated microbial communities. Five lines and two generations of each crop were subjected to high-throughput amplicon sequencing of the 16S rRNA and internal transcribed spacer (ITS) regions. Bacterial and fungal communities differed most by crop type (30% and 47% of the variance), while generation explained an additional 10% and 15% of the variance. The offspring (i.e., generation harvested in 2016 at the same location) exhibited a higher number of common amplicon sequence variants (ASVs) and less variability in microbial composition. Additionally, in every sample analyzed, a "core microbiome" was detected consisting of 5 bacterial and 12 fungal

[1] Moreira, Z.P.M., Helgason, B.L., and Germida, J.J. 2021. Crop, genotype, and field environmental conditions shape bacterial and fungal seed microbiomes. Can. J. Microbiol. **67**(2): 161–173. This is an open-access article distributed under the terms of the Canadian Science Publishing.

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ASVs. Our results suggest that crop, genotype, and field environmental conditions contributed to the seed-associated microbial assemblage. These findings not only expand our understanding of the factors influencing the seed microbiome but may also help us to manipulate and exploit the microbiota naturally carried by seeds.

3.3. Introduction

About 370,000 species (or 97 %) of vascular plants replicate through seeds (Christenhusz and Byng 2016; Royal Botanic Gardens 2017). In addition to their primary role in reproduction, seeds also aid the adaptation, persistence, and distribution of plants in different environments (Nelson 2018). Like other plant organs, seeds are colonized by microorganisms and provide specific microhabitat conditions for microbial life (Berg et al. 2014b; Wassermann et al. 2019). In agricultural systems the plant microbiome offers the potential of improving crop production and yield, through protection against abiotic and biotic stresses such as climate change, nutrient acquisition, pests, and pathogens (van der Heijden and Hartmann 2016; Rodriguez et al. 2019; Singh et al. 2019). Plant-associated microorganisms can be acquired indirectly from the surrounding environment or directly from the parent, creating a vertical transmission pathway for microbial inheritance. These microbes can be either transient or persistent during the different stages of plant development (Cope-Selby et al. 2017; Shade et al. 2017; Sánchez-López et al. 2018a). Vertical transmission to seeds occurs through the plant vascular system, from the stigma, or by contact with other organs such as fruits and flowers. Horizontal transmission is attributed to the soil, air, insects, or via contact with tools and infrastructure at harvest and post-harvest stages (Maude 1996; Mitter et al. 2017; Klaedtke et al. 2016).

Even though seeds act as an initial source and reservoir of microbes, studies regarding the seed microbiome are underrepresented in the literature (Wassermann et al. 2019) when compared with other plant habitats such as the rhizosphere (Rodriguez et al. 2019) or phyllosphere (Stone et al. 2018). To date, most seed microbiome studies have focused on the analysis of culturable microbiota, endophytes, the comparison of microbial communities among different cultivars, geographical locations, or agricultural practices; whereas seed microbiome persistence among crops and across generations remains largely unknown (Khalaf and Raizada 2016; Truyens et al. 2015; Sánchez-López et al. 2018b). Similarly, bacteria have received more attention than fungi and reports considering both microbiomes (Barret et al. 2015; Links et al. 2014) are scarce.

Cereals, oilseeds, and legumes are staple crops that significantly contribute to global food supply due to their caloric and protein content, agronomic traits, and cost of production. Production of wheat, canola, and lentil in Canada, reached 31.8, 20.3, and 2.1 million tonnes in 2018, respectively (Statistics Canada 2020a). However, production levels around the world will need to double to feed a growing global population expected to reach 9 billion by 2050 (Fischer et al. 2014; Dawson et al. 2016). Considering that these three crops are important for food security and the agricultural production sector and that they represent a broad variety of plant species, a deeper study of the microbial communities associated with their tissues is necessary to understand the role and contribution of these microorganisms in plant fitness, which could lead to a more productive and sustainable agriculture.

We hypothesized that the crop, genotype, and environmental location (i.e., seed origin) would influence seed microbiome assemblage. Thus, seeds harvested from different field locations and years would exhibit more variability in microbial community composition than seeds grown under the same conditions, but the host plant would still impact the community structure of the seed microbiota. To address this hypothesis, we used high-throughput amplicon sequencing of 16S rRNA and internal transcribed spacer (ITS) regions to characterize the bacterial and fungal seed microbiome of five *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines across two generations. Increasing our knowledge of assembly and dynamics of the seed microbiome will lead to better strategies for manipulating the plant microbiome through breeding, application of biotechnology tools such bio-inoculants, and crop production practices.

3.4. Materials and methods

3.4.1. Seed Source

For each crop type, five lines and two generations were analyzed. Lines AAC Penhold, AC Barrie, Frontana, Red fife, and Sumai 3 in *T. aestivum*; lines NAM 0, NAM 13, NAM 17, NAM 37, and NAM 72 in *B. napus*; and lines CDC KR-1, CDC Asterix, CDC Marble, CDC QG-3, and Schwarze Linse in *L. culinaris* (Table A.1, Appendix A). The seeds from the first generation, also known as parents, came from different sources (i.e., location, harvesting year; Table A.2, Appendix A), whereas their offspring was planted to and harvested from one location (Saskatoon, Saskatchewan, Canada) in 2016. For the parents, three technical replicates of each seed batch were profiled, whereas for the offspring, separate trials were conducted for each crop in a randomized complete block design experiment with three replicates. Plot size was 4m × 2m in the *T. aestivum*

and *L. culinaris* trials and 6m × 2m in the *B. napus* trial. Only *B. napus* seeds were pre-treated with fungicide and insecticide (HELIX XTra® ; Syngenta, Guelph, Canada) as per normal agricultural production practices in Canada. *Triticum aestivum* and *L. culinaris* were planted on 19 May and harvested on 25 August. Meanwhile, *B. napus* was planted on 27 May and harvested from 10 to 22 September. None of the field plots exhibited visible signs of fungal diseases or insect pests (personal communication, Steve Ryu, Plant Phenotyping and Imaging Research Centre). The harvesting process was carried out using a plot combine harvester (Zürn 110; Obergurig, Germany). Seeds were stored in paper bags at 21°C (room temperature) until analysis.

3.4.2. DNA extraction from seed-associated epiphytic microbiota

Total epiphytic community DNA was extracted using a modified protocol as described previously (Rastogi et al. 2010; Martins et al. 2013) in which 5g of each replicate were soaked in a 25mL wash solution (20 $mmolL^{-1}$ Tris-HCl, 10 $mmolL^{-1}$ EDTA, and 0.024% Triton) with shaking (150 r/min) at room temperature for 15 min. The washes were filtered using a 0.22 µm membrane (Pall, Mexico) and the DNeasy® PowerWater Kit (Qiagen, Hilden, Germany) was used to remove the DNA from the membranes. DNA quantity and quality were measured using a Qubit® Fluorometer (Invitrogen, Carlsbad, California, USA).

3.4.3. DNA extraction from seed-associated endophytic microbiota

Total endophytic community DNA was extracted from surface-disinfected seeds (65% ethanol, 5 min; 1.2% sodium hypochlorite solution, 5 min; rinsed with sterile distilled water) using the DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany) (Compant et al. 2011; Johnston-Monje and Raizada 2011).

3.4.4. High-throughput amplicon sequencing

Amplicon libraries were prepared following the Illumina MiSeq System Handbook (Illumina 2013). The primer sets with Illumina adapters were 342F and 806R (Mori et al. 2014) and ITS1-F_KYO1 and ITS2-R_KYO2 (Toju et al. 2012), which target the 16S rRNA and the ITS regions for bacteria and fungi, respectively. The polymerase chain reaction (PCR) for the 16S rRNA gene amplification was performed in a 20µL volume containing a 0.1 $mmolL^{-1}$ concentration of each primer (IDT®, Coralville, Iowa, USA), 0.625 U of DreamTaq Hot Start DNA Polymerase (Thermo Scientific, Carlsbad, California, USA), 1.25× DreamTaq buffer, a 0.25 $mmolL^{-1}$ concentration of each dNTP (Invitrogen, Carlsbad, California, USA), and 5-15 ng of genomic DNA

using the following conditions: 5 min at 95°C; 35 cycles of 30 s at 95°C, 45 s at 54°C, and 1 min at 72°C; 7 min at 72°C in a T100™ Thermal cycler (Bio-Rad Hercules, California, USA). For the ITS region amplification, the reaction was performed using the HotStarTaq® Master Mix Kit (Qiagen, Hilden, Germany) with an annealing temperature of 51°C. PCR products were visualized on a 1.2% E-Gel™ Agarose Gel with SYBR™ (Invitrogen, Carlsbad, California, USA). The index PCR was prepared using the KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, Massachusetts, USA) and the Nextera XT Index kit v2 (Illumina Inc., San Diego, California, USA). PCR products were purified with NucleoMag magnetic beads (NucleoMag® NGS Clean-up and size select; Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. Negative control PCR products were included in the libraries.

3.4.5. Bioinformatics and Statistical Analysis

Before processing Illumina sequencing data, an overall quality assessment of the raw reads was carried out using FastQC version 0.11.7 (Andrews 2010). Primers were removed using cutadapt version 2.1 (Martin 2011), imported into QIIME2 version 2019.1 (Bolyen et al. 2019), filtered, and assembled into amplicon sequence variants (ASVs) in Deblur (Amir et al. 2017) and DADA2 (Callahan et al. 2016) for bacteria and fungi, respectively. Deblur includes a filtering step, which removes chimeras. ASVs were classified using a 342F- and 806R-trained V3-V4 SILVA database version 132 (Quast et al. 2013) and UNITE database version 7.2 (Abarenkov et al. 2010).

The Vegan R package (Oksanen et al. 2019) was used to convert ASV abundances to relative abundance, and the Phyloseq package (McMurdie and Holmes 2013) to estimate the α diversity through the measurement of the Chao1 and inverse Simpson's indexes. To assess α diversity among crops and lines as well as between generations, linear mixed models (fixed effects: crop + generation + crop × generation, random effect: line), using the lme4 R package, were fit (Bates et al. 2019). Statistical significance was assessed using the Type III sums of squares with Satterthwaite's method in the lmerTest R package (Kuznetsova et al. 2019) followed by Tukey's post hoc tests using the multcomp R package (Hothorn et al. 2020) (R version 3.6.0). For subsequent analyses of individual crops, models considering the variable line as fixed factor were implemented.

Principal coordinate analysis (PCoA) was conducted on Hellinger transformed data to assess the microbial community structure distribution (β diversity) using Bray-Curtis dissimilarity

followed by permutational analysis of variance (PERMANOVA) evaluating all factors considered in this study and their interactions via the PC-ORD statistical package version 6.08 (McCune and Mefford 1999).

The analysis of composition of microbiomes (ANCOM) (Mandal et al. 2015) was used to identify taxa with differential abundance among crops and between generations. For crops comparisons, only the offspring results are displayed, since they were harvested from the same location and year. ANCOM plugin is incorporated into QIIME2.

We assigned the term "common" to the ASVs found in at least one (but not all) of the replicates of each treatment or subset examined and the term "core" to ASVs found in every replicate of the treatment or subset examined. The relative abundance data in Figs. 3.3 and 3.4 are aggregated to and presented at the highest taxonomic resolution assigned in SILVA and UNITE databases (at 99% similarity), i.e., order for bacteria and genus for fungi, to illustrate differences in community composition across treatment subsets. Members of the "core microbiome" in our study, were identified to genera and species level using the NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

3.4.6. Data Deposition

The raw sequence files supporting this article were deposited in the NCBI sequence read archive (SRA) under the accession numbers: SAMN12685051-SAMN12685229, SAMN15012655-SAMN15012657, SAMN15013312-SAMN15013314, and Bioproject ID PRJNA563687.

3.5. Results

A total of 2,706 bacterial and 2,092 fungal ASVs were generated from 1,547,374 and 4,424,272 reads, respectively, after sequencing the epiphytic library. α-Diversity measurements of the epiphytic microbiome revealed that the estimated richness (Chao1 index) was significantly different ($p < 0.05$) between generations for both bacteria and fungi; however, these differences were crop-dependent (Fig. 3.1A and C). In contrast, differences in diversity (Inverse Simpson's index) were observed among crops ($p < 0.05$) but not between generations (Fig. 3.1B and D). Based on these results, a subsequent analysis of individual crops was carried out. Variations in bacterial α-diversity estimators were found across lines and between generations in two of the three crops analyzed, with decreases in richness in *L. culinaris* and decreases in diversity in *T. aestivum*

offspring (Fig. 3.1A and C; Table B.1, Appendix B). Similarly, differences in fungal diversity were found across lines in all three crops but only in the parents (Fig. 3.1B and D; Table B.1, Appendix B).

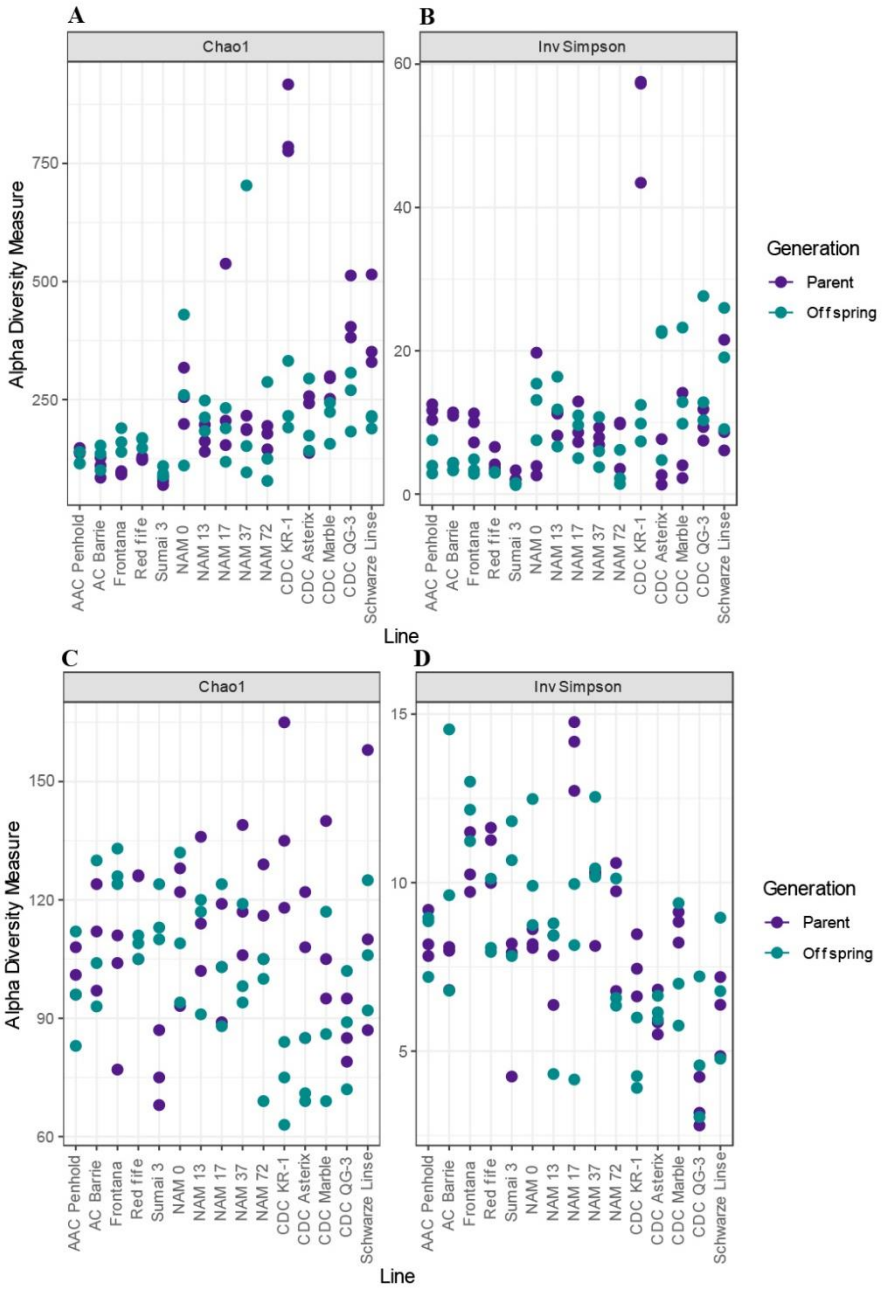


Figure 3.1 Estimated richness (Chao1 index) and diversity (Inverse Simpson's index) of microbial communities associated with seeds. Ninety seed samples belonging to 2 generations, 3 crops, 15 lines (5 per crop), and 3 replicates were assessed. Bacterial richness **(A)** and diversity **(B)** through the V3-V4 region of the 16S rRNA gene. Fungal richness **(C)**, and diversity **(D)** using the internal transcribed spacer (ITS) region.

β-Diversity analysis based on PCoA ordination of the Bray-Curtis dissimilarity displayed distinctly clustered communities between crop and generations for both bacteria (Fig. 3.2A) and fungi (Fig. 3.2B), with a much greater separation of clusters between crop type than between generation. Similarly, a greater separation between generations was observed in *L. culinaris.* PERMANOVAs evaluating all factors and their interactions corroborated that crop type, generation, and their interaction significantly influenced microbial community composition. Crop type captured most of the variance in both bacterial and fungal communities with 30% and 47% ($p < 0.001$) followed by the interaction of crop type with generation 21% ($p < 0.001$). Generation alone explained 10% and 15% ($p < 0.001$) of the variance in community structure, respectively. In *T. aestivum* and *L. culinaris*, the interaction of line × generation comprised the largest source of variation for both microbial communities with approximately 40% of variance for bacteria and 60% for fungi ($p < 0.001$). In *B. napus* generation explained 25% and 42% ($p < 0.001$) of the variance for bacterial and fungal communities. When the generations were assessed separately, line was a significant determinant of differences in community structure in all crops, accounting for up to 74% of variation in bacterial and 85% in fungal communities in the parents. Interestingly, in the offspring, the microbial communities were more consistent across lines within the crops. For instance, in *T. aestivum*, line explained 74% of the bacterial variance in the parents and only 42% in offspring (Table 3.1), whereas in *B. napus* and *L. culinaris*, bacterial variance was not explained by line in the offspring.

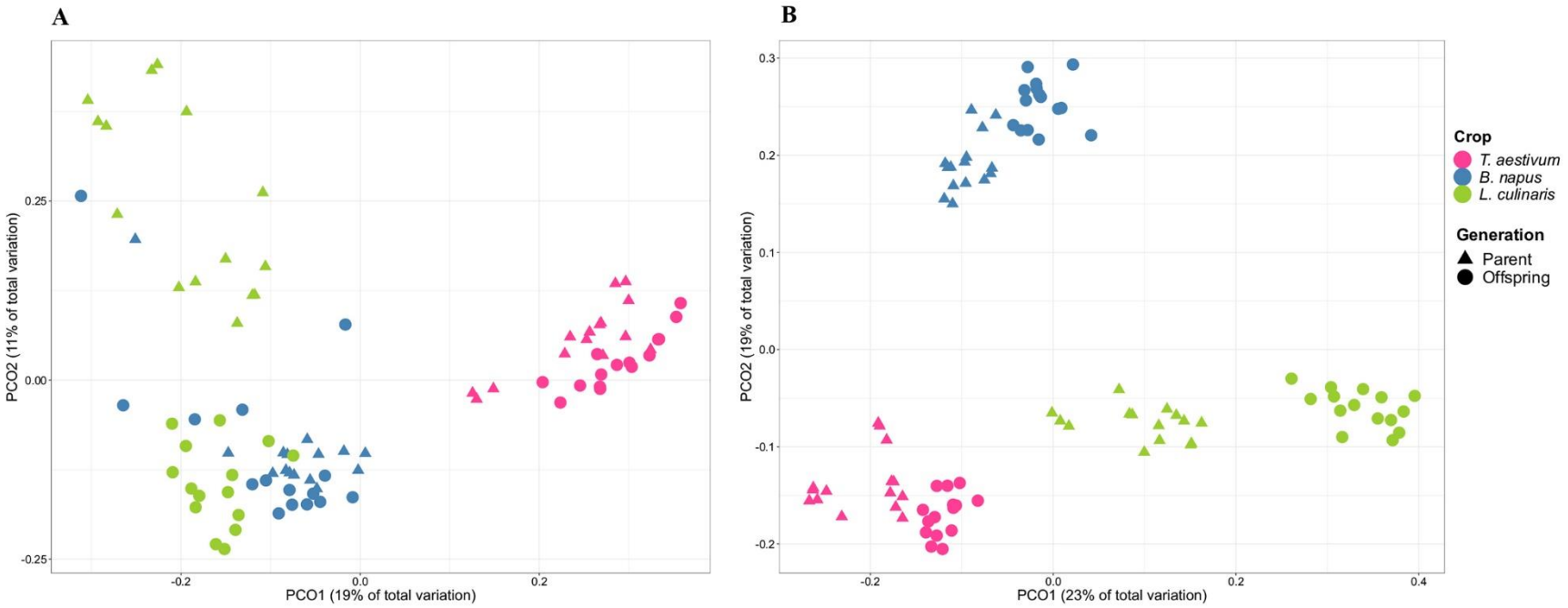


Figure 3.2 Principal coordinate analysis (PCoA) based on Bray-Curtis index of bacterial **(A)** and fungal **(B)** amplicon sequence variants (ASVs) from seed samples of 2 generations, 3 crops, and 15 lines.

Table 3.1 PERMANOVA (permutational analysis of variance) of β-diversity estimators for bacterial and fungal communities associated with *Triticum aestivum*, *Brassica napus*, and *Lens culinaris*.

	16S rRNA		ITS1	
	p	**Variance (%)**	***p***	**Variance (%)**
All crops				
Crop	0.000200	30.34	0.000200	46.81
Generation	0.000200	10.26	0.000200	14.96
Crop × Generation	0.000200	21.38	0.000200	20.93
T. aestivum				
Line	0.000200	28.37	0.000200	20.37
Generation	0.000200	30.46	0.000200	42.77
Line × Generation	0.000200	42.28	0.000200	57.90
Line (Parents)	0.000200	73.84	0.000200	77.96
Line (Offspring)	0.000200	41.43	0.000200	45.86
B. napus				
Line	0.228600	2.01	0.016000	11.21
Generation	0.000200	24.98	0.000200	41.66
Line × Generation	0.004600	16.15	0.000200	35.68
Line (Parents)	0.000200	28.23	0.000200	73.30
Line (Offspring)	0.231600	3.94	0.037200	8.66
L. culinaris				
Line	0.018800	9.43	0.001400	18.60
Generation	0.000200	33.61	0.000200	41.60
Line × Generation	0.000200	44.17	0.000200	67.92
Line (Parents)	0.000200	58.44	0.000200	85.52
Line (Offspring)	0.145000	4.65	0.000600	22.33

To further analyze the microbial community composition, the "common" amplicon sequence variants (i.e., ASVs found in at least one of the replicates of each treatment) were explored in all crops and generations. The common bacterial and fungal ASVs ranged from 4% to 21%, and the highest number was found in the offspring with up 141 common ASVs (Table 3.2). In addition, we found a "core microbiome" when the whole dataset was examined, in which a shared set of microorganisms was observed in every sample of *T. aestivum*, *B. napus*, and *L. culinaris* (i.e., in all lines, generations, and replicates). This core included 5 bacterial and 12 fungal ASVs. Core bacterial taxa were assigned to *Pantoea agglomerans*, *Curtobacterium flaccumfaciens*, *Sphingomonas* sp., *Rhodococcus fascians*, and *Rathayibacter tritici* species. Fungi comprised members of *Alternaria* spp., *Mycosphaerella tassiana*, *Vishniacozyma* spp., *Fusarium acuminatum*, *Filobasidium* spp., *and Dioszegia hungarica* species (Table 3.3). The core microbiomes associated with all samples within an individual crop type contained a larger number of taxa, ranging from 5 to 30 ASVs across both generations and 5 to 42 ASVs when generations were examined separately (Table C.1 and C.2, Appendix C).

Table 3.2 Summary of amplicon sequence variants (ASVs) found in the seed microbiome of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines across two generations.

		Parents	Offspring	Parents + Offspring
Bacteria				
	All crops			
	Total	2464	1564	2706
	[a]Common	232 (9%)	234 (15%)	392 (14%)
	[b]Core	5	12	5
	T. aestivum			
	Total	421	413	569
	[a]Common	42 (10%)	87 (21%)	110 (19%)
	[b]Core	19	34	16
	B. napus			
	Total	1164	1154	1603
	[a]Common	96 (8%)	127 (11%)	207 (13%)
	[b]Core	30	23	16
	L. culinaris			
	Total	2105	909	2360
	[a]Common	86 (4%)	141 (15%)	243 (10%)
	[b]Core	11	37	9
Fungi				
	All crops			
	Total	1346	1123	2092
	[a]Common	118 (9%)	111 (10%)	172(8%)
	[b]Core	13	20	12
	T. aestivum			
	Total	457	498	785
	[a]Common	53 (12%)	70 (14%)	89 (11%)
	[b]Core	26	42	22
	B. napus			
	Total	563	528	903
	[a]Common	61 (11%)	65 (12%)	87 (10%)
	[b]Core	37	35	30
	L. culinaris			
	Total	691	442	971
	[a]Common	32 (5%)	57 (13%)	73 (8%)
	[b]Core	20	28	17

[a] ASVs found in not all but at least one of the replicates of each sample examined.
[b] ASVs found in every sample and replicate examined.

Table 3.3 Members of the shared set of microorganisms (i.e., core microbiome) found in all lines, generations, and replicates of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris.*

Core Bacterial ASVs	SILVA Database	Blast ID
8bf175f329d16e4aa732cf2b32279df3	Enterobacteriaceae	*Pantoea agglomerans*
a6580129a3ace1a83e446dca31617824	Microbacteriaceae	*Curtobacterium flaccumfaciens*
cce70d5962057843ac2fbadc53741e55	Sphingomonadaceae	*Sphingomonas* sp.
c9de781a5fcb7e51394be942835afc3	Nocardiaceae (*Rhodococcus* sp.)	*Rhodococcus fascians*
94dbd0eda2f2563cdf95d7cd68e2ec4b	Microbacteriaceae (*Rathayibacter* sp.)	*Rathayibacter tritici*
Core Fungal ASVs	**UNITE Database**	**Blast ID**
63a75f273959682f2af9bbd1037146dd	*Alternaria* sp.	*Alternaria alternata*
808e6ff2ae17bba825851375849fdc72	*Mycosphaerella tassiana*	*Cladosporium herbarum* (anamorph of *Mycosphaerella*)
2119b24c0bba7c01ab5ed649948d64d0	*Chalastospora gossypii*	*Alternaria* sp. (var. polymorpha)
8b0dfcd895be861832041188cefb49e1	*Vishniacozyma victoriae*	*Vishniacozyma victoriae*
d0f63ae4057c8d978e554cc723b10414	*Vishniacozyma victoriae*	*Vishniacozyma victoriae*
8b41e27a4be11eb0b559db7ded7dd91b	*Vishniacozyma victoriae*	*Vishniacozyma victoriae*
f034ec725857a24818422228b7fe8b54	*Fusarium* sp.	*Fusarium acuminatum*
f005bb8a7befe140577df369f966ef0e	*Filobasidium* sp.	*Filobasidium oeirense*
f64349490a3d8a5573ec60e996649023	*Vishniacozyma* sp.	*Vishniacozyma tephrensis*
fb3ce85eb31eb08af3811269d7d4f729	*Filobasidium magnum*	*Filobasidium magnum*
96f24862bb0534f504648d677d32cba7	*Dioszegia hungarica*	*Dioszegia hungarica*
e1f579c16ab012b62ecee5df562e21d9	*Mycosphaerella tassiana*	*Cladosporium herbarum* (anamorph of *Mycosphaerella*)

Using ANCOM, we also identified ASVs that were differentially abundant between generations and crops. For example, across generations, the relative frequency of members of the bacterial class Gammaproteobacteria significantly increased in *B. napus* and *L. culinaris* offspring. Similarly, members of the fungal class Leotiomycetes were more abundant in *L. culinaris* offspring when compared with the parents. In contrast, lentil parents harbored a higher relative abundance of Eurotiomycetes (Tables D.1 and D.2, Appendix D). Notably, Gammaproteobacteria also had a significantly higher frequency in *T. aestivum* than in *B. napus* and *L. culinaris.* Dothideomycetes fungi were more abundant in *B. napus* than in *T. aestivum* and *L. culinaris.* Meanwhile, Leotiomycetes and Sordariomycetes fungi were more abundant in *L. culinaris* than in *B. napus* and *T. aestivum* (Tables D.3 and D.4, Appendix D).

Fig. 3.3 shows that the seed microbiome of *T. aestivum*, *B. napus*, and *L. culinaris* predominantly contained members of the bacterial orders Enterobacteriales and Pseudomonadales, which belong to the Gammaproteobacteria class. Similarly, Fig. 3.4 shows a higher content of *Sclerotinia* in lentil seeds harvested in 2016; this genus is classified in the Leotiomycetes class. Fluctuations in relative abundance among lines were notably observed in all crops and generations. However, in the generation designated as "parents", differences among lines were more pronounced (Fig. 3.3, Fig. 3.4; Table 3.1).

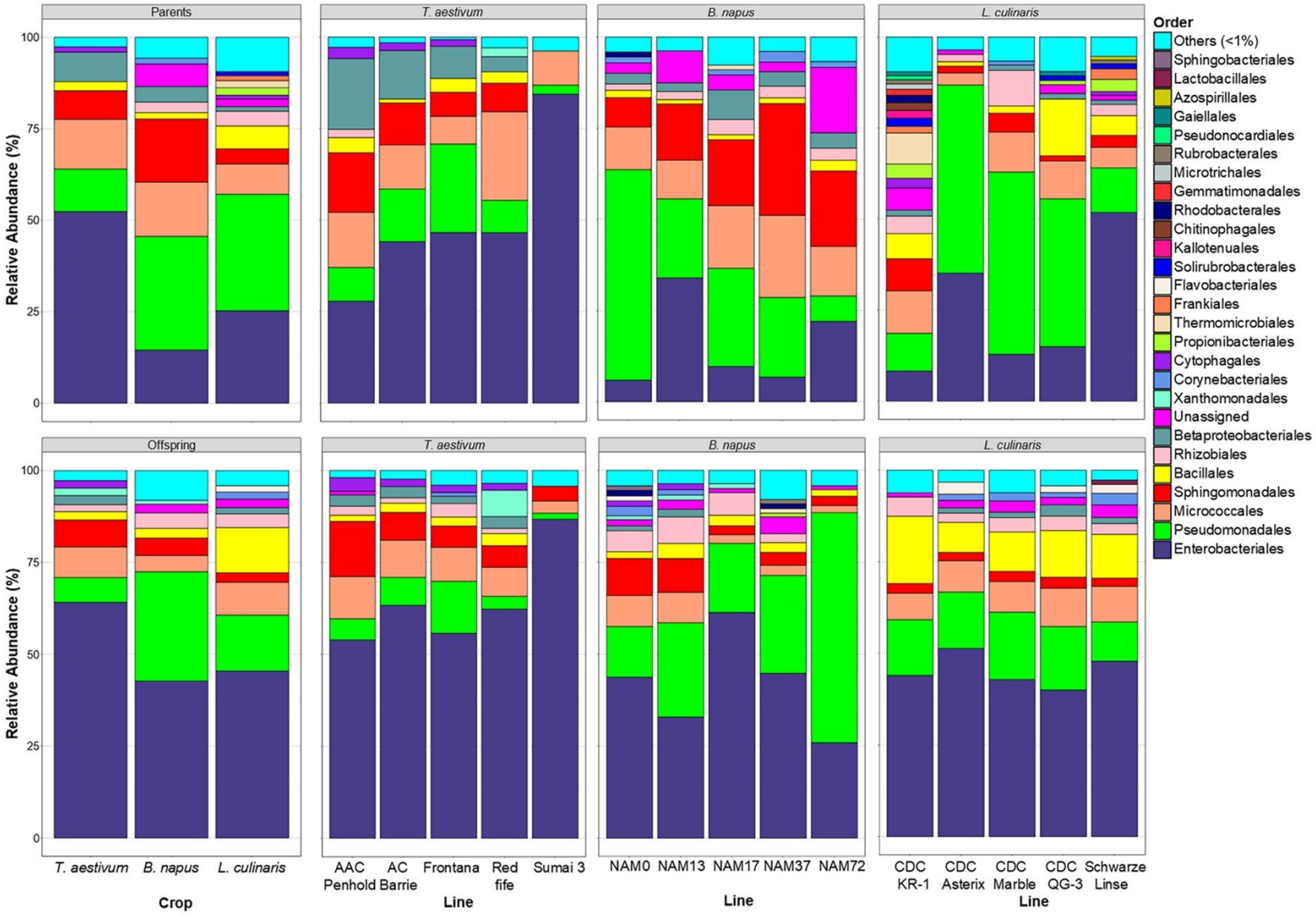


Figure 3.3 Relative abundance of the dominant bacterial orders in the seed microbiome of *Triticum aestivum*, *Brassica napus*, *and Lens culinaris* lines across two generations. Data are shown as mean value across three replicates.

Figure 3.4 Relative abundance of the dominant fungal genera in the seed microbiome of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines across two generations. Data are shown as mean value across three replicates.

Attempts to characterize the endophytic microbiome were unsuccessful as the sequences obtained originated mostly from plant plastid or mitochondria (Fig. E.1, Appendix E).

3.6. Discussion

Our findings indicate that genetic and field environmental conditions (i.e., location and harvest year) factors are key drivers of seed microbiome assemblage in the three crops assessed. Crop type accounted for the largest source of variation for both bacterial (30%) and fungal communities (47%), suggesting that plant genotype strongly influence microbial community composition. *Triticum aestivum*, *B. napus*, and *L. culinaris* are taxonomically distant, belonging to different plant orders, which may explain the high degree of genotype specificity. This is supported by the fact that all offspring were cultivated at the same location (Saskatoon, Saskatchewan, Canada) in 2016, and differences in microbial community composition persisted across crop types and lines. Zilber-Rosenberg and Rosenberg (2008) and Berg et al. (2016) postulated that differences such as those observed in our study can be explained by the co-evolution of plants and microorganisms. We have no direct knowledge or data to support this idea of co-evolution, but it is possible and best illustrated in mycorrhizal fungi-host plant associations (Cairney 2000; Prasad et al. 2017). Future research should consider this phenomenon in plant breeding programs. The genotype specificity found in our samples may also be linked to canopy characteristics, which means each crop genotype provides numerous microhabitats with their own potentially unique microbial interactions (Nakamura et al. 2017; Cregger et al. 2018). Microbes are transmitted through the flowers, and microbial communities colonizing petals, pistils, or pollen may be incorporated into the seeds (Hodgson et al. 2014; Mitter et al. 2017). Moreover, microorganisms transported by insects visiting plants during flowering, soil, and air may influence seed microbiome assemblage (Rodríguez et al. 2020; Prado et al. 2020). Seed biochemical composition and consequently the resources available for the seed-associated microbiota could also explain differences among crops (Sliwinska and Bewley 2014; Torres-Cortés et al. 2019). *Triticum aestivum* and *L. culinaris* seeds are rich in carbohydrates and proteins, whereas *B. napus* is rich in oil and other compounds, including glucosinolates, phytates, and phenols (Shewry and Halford 2002; Anwar et al. 2015; United States Department of Agriculture 2019).

Generation explained 10% and 15% of the variance in bacterial and fungal seed microbiomes, respectively. Generation was at least partly related to the environment in which the samples were planted to and harvested from. The parents came from different sources (location,

harvesting year) and their offspring were produced in one location in 2016. Significant differences in richness and diversity between parents and offspring were found in most lines analyzed, reflecting that environmental conditions such as field location, and potentially, agricultural management practices are contributing factors in seed-associated microbial assemblage. Similar results were reported by Klaedtke et al. (2016) and Rochefort et al. (2019) in the seed microbiome of *Phaseolus vulgaris* L. and *B. napus* collected from different locations and years.

More generally, bacterial and fungal richness were determined by both crop type and generation, whereas diversity was driven only by the crop type. More specific patterns were observed with crop type. For example, in *T. aestivum* and *L. culinaris*, decreases in bacterial diversity and richness were detected in the offspring, which may be related to the fact that all offspring were harvested from the same location and year (2016) thereby reducing the exposure of the seeds to exogenous organisms from a greater variety of environments. Seed exposure could also explain patterns observed in *B. napus* parents, in which no significant differences in α diversity were observed. *Brassica napus* parents were propagated in hoop tents and not in the field, thus reducing environmental effects.

Lens culinaris CDC KR-1 was obtained from a seed trading company; hence, the seed lot included samples from different field locations. Major differences were observed in the intergenerational microbiome composition of CDC KR-1, which exhibited not only the highest bacterial richness but also the highest bacterial diversity found among all of our samples. This comparatively high diversity was observed in the parent seed lot, but not in the offspring, supporting our hypothesis that seeds harvested from different field locations and years would exhibit more variability in microbial community composition. Less differences among lines and a higher number of common ASVs were detected in all crops' offspring. This also implies that the field environmental location or source is influential in the seed microbiome assemblage (Shade et al. 2017; Berg and Raaijmakers 2018).

Although the seed samples analyzed here belong to different crops and lines, and were produced under different environmental conditions (i.e., location, harvesting year), 5 bacterial and 12 fungal ASVs persisted in every single sample and replicate examined (Table 3.3). This commonality suggests that there may be a “core microbiome” associated with the seed of multiple crop species across a variety of conditions. The core included members of bacterial and fungal

taxa, such as *Pantoea agglomerans*, *Sphingomonas* sp., and *Vishniacozyma* spp. Some strains of these genera are known to have beneficial effects on plant fitness, suggesting that they may have important roles in plant development. For instance, Links et al. (2014) and Town et al. (2016) reported that *P. agglomerans* isolate 4, a member of the seed epiphytic microbiome in *T. aestivum*, exhibited antagonistic properties toward the pathogenic fungi *Alternaria* sp. Similarly, Khalaf and Raizada (2020a) reported that *P. agglomerans* strains EKM10T, EKM20T, EKM21T, and EKM22T isolated from biogels (mucilage) coating *Cucumis sativus* L. and *Cucumis melo* L. seeds suppress the growth of soil-borne oomycete and fungal phytopathogens *in vitro*. Moreover, *Sphingomonas* sp. strains CT25 and CT33 isolated from *Oryza sativa* seeds produced indole acetic acid, an auxin phytohormone involved in plant growth promotion (Ruiza et al. 2011). These commonalities among members of the core microbiome found in our study and isolates with known beneficial properties suggest possible benefits, but it is well known there is strain specificity (i.e., biovar) of some traits and further research is needed to clarify beneficial (or detrimental) impacts of these organisms (van Brussel et al. 1990; Andrews and Andrews 2017; Allito et al. 2020). Even though potentially beneficial species were found in the core, pathogens including *Curtobacterium flaccumfaciens, Rhodococcus fascians, Alternaria* spp., *Mycosphaerella tassiana*, and *Fusarium acuminatum* were also identified. *Curtobacterium flaccumfaciens* has been reported in *Phaseolus vulgaris* and *Glycine max* seeds (Soares et al. 2018; Tegli et al. 2017). *Alternaria* spp. have been described as part of the core seed-associated microbiota in several plant species, including *T. aestivum, B. napus*, *O. sativa*, *Raphanus sativus*, and others (Links et al. 2014; Rezki et al. 2018; Eyre et al. 2019).

Variations in the relative abundance of bacterial and fungal taxa between generations and crops were observed in our study. Links et al. (2014) also observed a predominant occurrence of Enterobacteriales and *Alternaria* in the seed microbiome of wheat. Meanwhile, Rochefort et al. (2019) reported that *B. napus* lines mainly contain members of Pseudomonadales, Sphingomonadales, and *Alternaria* taxa. Thus, the microbial composition found in our *T. aestivum* and *B. napus* samples is consistent with previous reports. Here, we provide the first insight into the *L. culinaris* seed microbiome.

Endophytic seed microbiomes have been reported for *B. napus* (Rybakova et al. 2017) and *T. aestivum* (Kuźniar et al. 2020b). Unfortunately, despite repeated attempts and testing of multiple methodologies (not reported), we were unable to detect substantial endophytic microbiomes.

Instead, using the methodology in our study, the overwhelming majority of sequences obtained from "endophytic" samples originated from plant plastids or mitochondria (*T. aestivum*: 1.8% plastids, 97.8% mitochondria; *B. napus*: 31.9% plastids, 68% mitochondria; *L. culinaris*: 0.3% plastids, 99.5% mitochondria). Plant genotype, inhibitors, or antimicrobial compounds may explain the low bacterial loads present in our samples, a finding observed previously by Robinson et al. (2016). According to Fouhy et al. (2016), primer choice and sequencing platform could also affect the bacterial composition results obtained from complex environments through the 16S rRNA gene sequencing.

In conclusion crop, genotype, and field environmental conditions influence the seed microbiome assemblage in agricultural crops. The presence of a core microbiota associated with each crop suggests that transmission, preservation, and recruitment of microorganisms are determined to some extent by the host. Characterizing the community structure of the seed microbiome informs our understanding of how different plants and their environment affect the identity and ecology of microorganisms carried on these vital organs. This new knowledge will support exploitation of the seed microbiome to optimize the plant microbiome and promote more sustainable agriculture.

4. ENVIRONMENT HAS A STRONGER EFFECT THAN HOST PLANT GENOTYPE IN SHAPING SPRING *BRASSICA NAPUS* SEED MICROBIOMES[2]

4.1. Preface

Results presented in Chapter 3 suggest both genetic and environmental factors are key drivers of seed microbiome assemblage in agricultural crops. However, the relative contribution of environmental factors versus host genotype is not clear. In this chapter, eight canola lines harvested from four site years were characterized to better understand the relative contribution of biotic and abiotic factors in the seed-associated microbiota assemblage.

4.2. Abstract

Seeds are reproductive units that transfer genetic information to the next generation and harbor microbial communities that may interact with a host plant at all stages of its development. Here we assessed the effect of the environment and plant genotype on the seed microbiome of eight spring *Brassica napus* lines harvested from four site years in Saskatchewan, Canada: one location each in 2016 and 2017 and two additional locations in 2017. Seed microbiomes were characterized using high-throughput amplicon sequencing of the bacterial 16S ribosomal RNA and fungal transcribed spacer (ITS) regions. Our results revealed that microbial communities were predominantly shaped by the environment, with location explaining 34% of bacterial and 43% of fungal total variance. Meanwhile, genotype had a smaller effect, accounting for only 9% of bacterial and 13% of fungal variance. The seed microbiome of *B. napus* predominantly contained members of Enterobacteriales and Pseudomonadales bacterial orders as well as Pleosporales and Capnodiales fungal orders. Additionally, common taxa including Enterobacteriales,

[2] Moreira, Z.P.M., Helgason, B.L., and Germida, J.J. 2021. Environment has a stronger effect than host plant genotype in shaping spring *Brassica napus* seed microbiomes. Phytobiomes J. doi:10.1094/PBIOMES-08-20-0059-R. This is an open-access article distributed under the terms of The American Phytopathological Society.
Authors' contributions: ZPMM contributed in the experimental design, sampling, laboratory analysis, processing and interpretation of data, and writing the manuscript. BLH contributed in the experimental design, interpretation of data, and manuscript revisions. JJG contributed in the experimental design, interpretation of data, and manuscript revisions.

Pseudomonadales, Micrococcales, Sphingomonadales, Pleosporales, Capnodiales, Tremellales, Filobasidiales, and Sporidiobolales, were detected across all site years. Our results demonstrate that the environment plays a dominant role in shaping spring *B. napus* seed microbiomes, with more subtle contributions related to host plant genotype. Information collected in this study may contribute to the development of novel and sustainable breeding and agricultural strategies that consider microorganisms carried by seeds.

4.3. Introduction

The plant microbiome contributes a significant level of genetic information that influences plant fitness (Zilber-Rosenberg and Rosenberg 2008; Sessitsch and Mitter 2015). Thus microbial communities can be either beneficial or deleterious for plant growth, development, and function, thereby affecting productivity and yield of agricultural crops (Andreote and Pereira e Silva 2017; Tosi et al. 2020). Each plant compartment including reproductive organs such as flowers (Arunkumar et al. 2019), fruits (Droby and Wisniewski 2018), and seeds (Nelson 2018) provides resources and habitat for a huge number of microbes, which colonize external and internal plant tissues. Seeds not only represent the starting point for a broad variety of plant species but also store and carry microbes that will continue to interact with the plant throughout its life cycle (Gopal and Gupta 2016). Thus, a plant's first microbial inoculum comes from the seeds.

Climate change, ecosystem degradation, and biodiversity loss significantly affect agriculture and food security; looking for sustainable strategies and practices is essential to ensure the global nutritional demand (Toju et al. 2018). Exploring plant microbiome functions is one approach that can mitigate adverse effects caused by biotic and abiotic stresses affecting plant fitness (Orozco-Mosqueda et al. 2018; Shinwari et al. 2019). Microbial communities associated with seeds are reported to suppress disease (Links et al. 2014; Jack and Nelson 2018; Khalaf and Raizada 2018) and directly promote plant growth (Walitang et al. 2017; Bergna et al. 2018) in cereals, fruits, vegetables, and other crops, which reveals their potential application in sustainable crop production. There is a paucity of information known about seed-associated microbiota, especially about their composition, dynamics, and assemblage (Shade et al. 2017; Wassermann et al. 2019).

Rapeseed (*Brassica napus*), soybean (*Glycine max*), and palm (*Phoenix dactylifera*) are the main sources of vegetable oil in the world (Liu et al. 2018). Low-erucic and low-glucosinolate

edible rapeseed varieties are known as “canola” (Canola Council of Canada, 2020a). Canola is considered a staple crop in the Canadian Prairies and crucial for global food security. Its use is not limited to human consumption; canola meal is utilized in animal feeds (Good et al. 2017) and as a feedstock for biodiesel production (Ge et al. 2018). Depending on the flowering time and local adaptation, canola cultivars grow during spring or winter seasons and can be annual or biannual (Schiessl et al. 2017). Production in Western Canada is based on spring canola, with the province of Saskatchewan the largest supplier, marketing about 11 million tonnes from 4,955,000 ha harvested in 2018 (Statistics Canada 2020a). Canola-producing areas are found in different soil and climatic zones, which represent a broad range of environments and ecological habitats (Government of Saskatchewan, 2019). Despite the agricultural and nutritional importance of this oilseed, there are few studies regarding its microbiome. In addition, the few reports available mainly focused on the canola root and rhizosphere microbiomes (Lay et al. 2018; Monreal et al. 2018; Floc’h et al. 2020; Cordero et al. 2020; Bazghaleh et al. 2020), whereas the seed microbiome is poorly understood.

In agricultural crops, assembly of the seed microbiome has been linked to host genotype (Adam et al. 2018; Raj et al. 2019), field management practices (Klaedtke et al. 2016), harvesting year (Rochefort et al. 2019), or anthropogenic pollutants (Truyens et al. 2016). Nevertheless, the relative contribution of environmental factors versus host genotype is mostly unknown. In a previous study (Moreira et al. 2021a) we profiled the seed microbiome of wheat (*Triticum aestivum*), canola (*Brassica napus*), and lentil (*Lens culinaris*) lines across two generations. We found that crop, genotype, and the environment were key drivers of the seed microbiome assemblage. To better understand the contribution of differences in environment, we profiled eight genetically different spring *B. napus* lines harvested from different locations in Saskatchewan, Canada. We hypothesized that the host genotype (i.e., line) would influence the *B. napus* seed microbiome structure and diversity. We further hypothesized that the collective influences of the environment (i.e., all sources of variation that are not genetic such as location and harvesting year), would have a stronger influence than genotype on the seed microbiome. To test these hypotheses, 16S ribosomal RNA (rRNA) and internal transcribed spacer (ITS) amplicon libraries from seed samples collected in four site years (one location in 2016 and 2017 and two additional locations in 2017) were prepared and sequenced. Understanding how environmental and genetic factors influence the assembly of the microbiota carried by seeds could provide insights into the

recruitment and transmission of microbial communities in plants. This understanding is essential for implementing novel and sustainable crop breeding and agricultural management practices.

4.4. Materials and methods

4.4.1. Lines and Experimental Design

Brassica napus seeds used in this study were harvested from four site years in Saskatchewan, Canada: one location (Saskatoon) in 2016 and 2017 and two additional locations (Melfort and Scott) in 2017. These three sites span a large portion of the local canola-growing regions and represent the different conditions under which canola is grown. Fields were located in the Dark Brown Soil Zone (Saskatoon, Scott; Typic Boroll) and in the Black Soil Zone (Melfort; Udic Boroll) (Table 4.1; Fig. F.1, Appendix F). At all field sites, *B. napus* lines were planted in a randomized complete block design experiment (plots 6m × 2m) with three replicates. Eight lines from a nested association mapping (NAM) population were selected to represent diversity within the set (Taye et al. 2020; Bazghaleh et al. 2020); NAM 0, NAM 13, NAM 17, NAM32, NAM 37, NAM 43, NAM 72, and NAM 94. Seeds planted at all fields came from the same source and were produced in hoop tents by Agriculture and Agri-Food Canada (Saskatoon, Saskatchewan, Canada). All seed samples analyzed in this study were stored in paper bags at 21°C (room temperature) until analysis. DNA extraction of seed samples collected from each site year was carried out approximately eight months after harvesting. Library preparation and Illumina sequencing were done in two batches for each gene. Samples from Saskatoon 2016, Saskatoon 2017, and Melfort 2017 comprised the first batch. Samples from Scott 2017 were processed separately due to a delayed harvest (Table 4.1; Fig. F.1, Appendix F) and subsequent delays in obtaining the seed.

Table 4.1 Saskatchewan, Canada field sites.

Location	Year	GPS Coordinates	Soil	Seeding	Harvesting	Precipitation during growing season (mm)	Mean Temperature (°C)
Saskatoon	2016	52°10’52.918”N, 106°30’10.587”W	Dark Brown Chernozem (Typic Boroll) Clay loam texture pH 7.5 Organic matter 5.1%	27 May	10-22 September	195.3	16.5
Saskatoon	2017	52°10’59.336”N, 106°30’53.654”W	Dark Brown Chernozem (Typic Boroll) Clay loam texture pH 7.5 Organic matter 5.1%	28-29 May	5-28 August	81.6	17.5
Melfort	2017	52°49’9.599”N, 104°35’46.852”W	Black Chernozem (Udic Boroll) Silty clay texture pH 6.4 Organic matter 8.2%[†]	19 May	9 September	92.5	16.6
Scott	2017	52°21’55.332”N, 108°52’32.555”W	Dark Brown Chernozem (Typic Boroll) Loam texture pH 5.7 Organic matter 5.8%[§]	20 June	17 October	106.2	13.7

[†]Foster et al. 2014.

[§]Bedard-Haughn et al. 2013 and Arcand et al. 2016.

4.4.2. DNA extraction and high-throughput amplicon sequencing

A modified protocol previously described by Rastogi et al. (2010) and Martins et al. (2013) was applied for DNA extraction of the seed epiphytic microbial communities. From each plot harvested, 5g of seeds were immersed in 25mL buffered wash solution containing 0.024% Triton, 10 $mmolL^{-1}$ EDTA, and 20 $mmolL^{-1}$ Tris-HCl in a 250 mL Erlenmeyer flask and shaken (150 r/min) for 15 min at room temperature. A 0.22 μm membrane (Pall, Mexico) was used to filter the solution followed by DNA extraction with the DNeasy® PowerWater Kit (Qiagen, Hilden, Germany) and quantification in a Qubit® Fluorometer (Invitrogen, Carlsbad, California, USA). Bacterial 16S rRNA and fungal ITS regions were amplified using the primer sets with Illumina adapters 342F and 806R (Mori et al. 2014) and ITS1-F_KYO1 and ITS2-R_KYO2 (Toju et al. 2012), respectively. PCR assays were carried out using DreamTaq Hot Start DNA Polymerase (Thermo Scientific, Carlsbad, California, USA) for 16S rRNA, and HotStarTaq® Master Mix Kit (Qiagen, Hilden, Germany) for ITS amplifications in a T100^{TM} Thermal cycler (Bio-Rad Hercules, California, USA) (Moreira et al. 2021a). NucleoMag magnetic beads (NucleoMag® NGS Clean-up and size select; Macherey-Nagel, Düren, Germany) were used to purify PCR products followed by barcoding with Nextera XT Index kit v2 (Illumina Inc., San Diego, California, USA) using KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, Massachusetts, USA). Libraries were sequenced in an Illumina MiSeq platform with the MiSeq Reagent kit v3 (600 cycles) for bacteria and MiSeq Reagent kit v2 (500 cycles) for fungi. Negative controls were included in the library preparation.

4.4.3. Bioinformatics and Statistical Analysis

Cutadapt version 2.1 was used for removing primers (Martin 2011). Sequences were processed in QIIME2 version 2019.10 (Bolyen et al. 2019), quality filtered, and assigned to amplicon sequence variants (ASVs) in Deblur (bacteria) (Amir et al. 2017) and DADA2 (fungi) (Callahan et al. 2016), in accordance with a standardized protocol developed for soil, rhizosphere, root, leaf, and seed microbiome analyses (Bazghaleh et al. 2020). A 342F- and 806R-trained V3-V4 SILVA database version 132 (Quast et al. 2013) and UNITE database version 8.0 (Abarenkov et al. 2010) were used to classify bacterial and fungal ASVs, respectively.

Data analyses were conducted in R version 4.0 using the Phyloseq package version 1.32.0 (McMurdie and Holmes, 2013) for α- and β-diversity metrics. Differences in α-diversity values

(Chao 1, inverse Simpson's) were tested using generalized linear models in the car package version 3.0.8 (Fox et al. 2020) followed by Tukey's post-hoc tests in the multcomp package version 1.4.13 (Hothorn et al. 2020). Multidimensional ordinations using principal coordinates analyses (PCoA) were performed on Bray-Curtis (Hellinger-transformed) dissimilarities to assess microbial community structure. To test whether seed microbial communities were significantly affected by environment or *B. napus* line, permutational multivariate analyses were carried out in the Vegan package version 2.5.6. (Oksanen et al. 2019) with the function adonis. Vegan was also used to obtain relative abundance of bacterial and fungal taxa. To identify taxa with differential abundance among environments the analysis of composition of microbiomes (ANCOM) in QIIME2 was applied (Mandal et al. 2015).

We use the term "core microbiome" to describe the ASVs present in every *B. napus* line and replicate analyzed in this study and in individual site years. Separate comparisons describe the taxonomy (class, order, family, genus) of bacterial and fungal ASVs detected within a given site year. These comparisons highlight taxa that were common across different environments without necessarily being the exact ASV match.

4.4.4. Data Deposition

Sequence data was deposited in the National Center for Biotechnology Information (NCBI) Sequence read archive (SRA). Bioproject ID PRJNA563687, accessions: SAMN14541915 to SAMN14542010.

4.5. Results

In total, 1,401,178 bacterial and 4,267,381 fungal reads were assigned to 3,000 and 1,765 ASVs, respectively (Table 4.2; Fig. G.1, Appendix G). α-Diversity estimators differed between 2016 and 2017 at the Saskatoon field location ($p < 0.05$). Specifically, bacterial and fungal diversity as well as bacterial richness were higher in 2016 (Fig. 4.1; Table H.1, Appendix H). Similarly, differences were detected between the three locations in 2017, where Melfort exhibited the highest richness and diversity for both bacterial and fungal microbial communities (Fig. 4.1; Table I.1, Appendix I). Comparisons within individual locations showed that *B. napus* line also significantly affected α diversity. For instance, in 2017 at Melfort and Saskatoon, line NAM 0 showed pronounced differences in fungal diversity when compared with other lines, but the patterns were

not consistent between locations or between years at the Saskatoon site (Fig. 4.1; Table H.1, Appendix H; Table I.1, Appendix I).

Table 4.2 Summary of amplicon sequence variants (ASVs) found in the seed microbiome of *Brassica napus* across years and locations.

	All site years	Saskatoon 2016 and 2017	2017 all sites	Saskatoon 2016	Saskatoon 2017	Melfort 2017	Scott 2017
Bacteria							
Total	3,000	1,806	2,555	1,299	1,063	2,165	434
[a]Core	2	4	2	18	6	20	9
Fungi							
Total	1,765	1,082	1,425	619	675	901	308
[a]Core	11	25	11	32	26	19	30

[a]ASVs found in every sample and replicate examined. Taxonomic assignment of the core ASVs can be found in bold in Table 4.4.

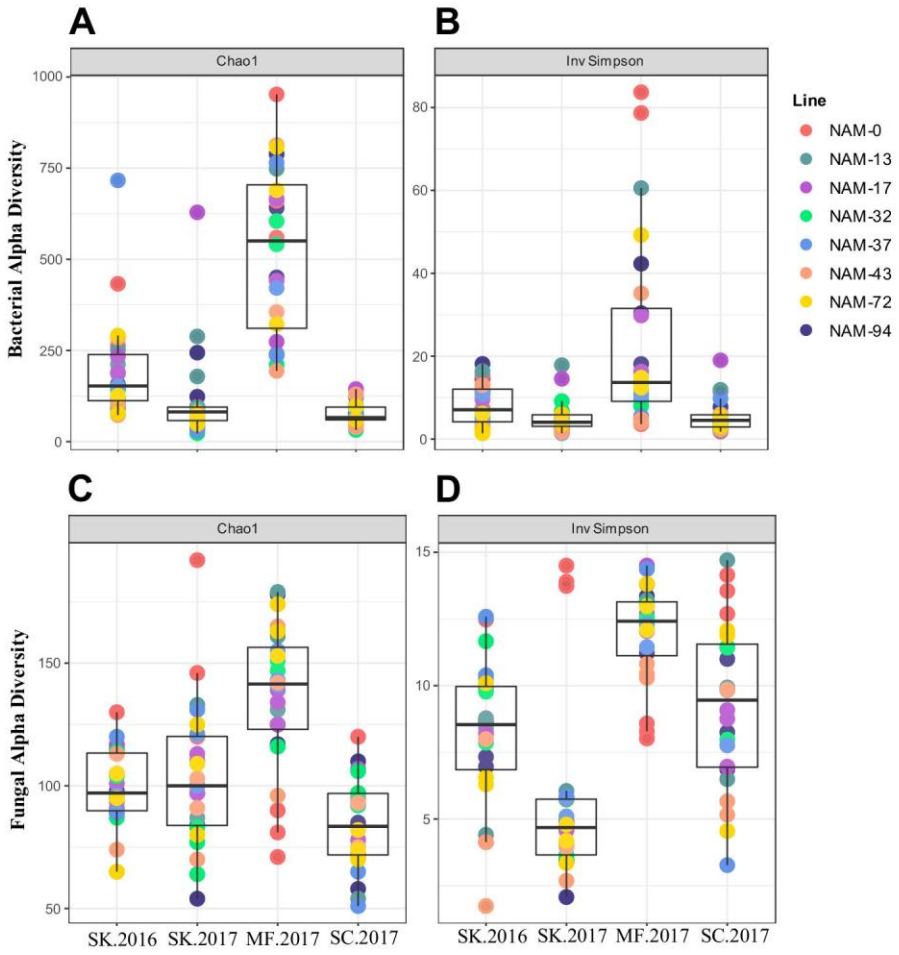


Figure 4.1 Box plots depicting α-diversity measures of bacterial and fungal communities associated with *Brassica napus* seeds harvested in Saskatchewan. NAM= nested association mapping population.

PCoA plots based on Bray-Curtis dissimilarity revealed a clear clustering of bacterial and fungal communities depending upon year (Fig. 4.2A and B) and location (Fig. 4.2C and D). Permutational multivariate analysis of bacterial and fungal communities corroborated that year and location (i.e., environmental conditions) ($p < 0.05$) were the main factors explaining the variation in the microbial community structure, with a significant interaction year × line and location × line for fungi but not for bacteria (Table 4.3). Thus, year explained 20% of the bacterial variance and 32% of the fungal variance. Similarly, location accounted for 34% of the bacterial variance and 43% of the fungal variance. Year and location interactions with line represented 18 and 26%, respectively, of the variance for fungal communities (Table 4.3). When all four site years were assessed separately, *B. napus* line accounted for 42 to 83% of the fungal variance within a given site year. Furthermore, Melfort 2017 was the only field where line did not explain bacterial community structure (Table 4.3).

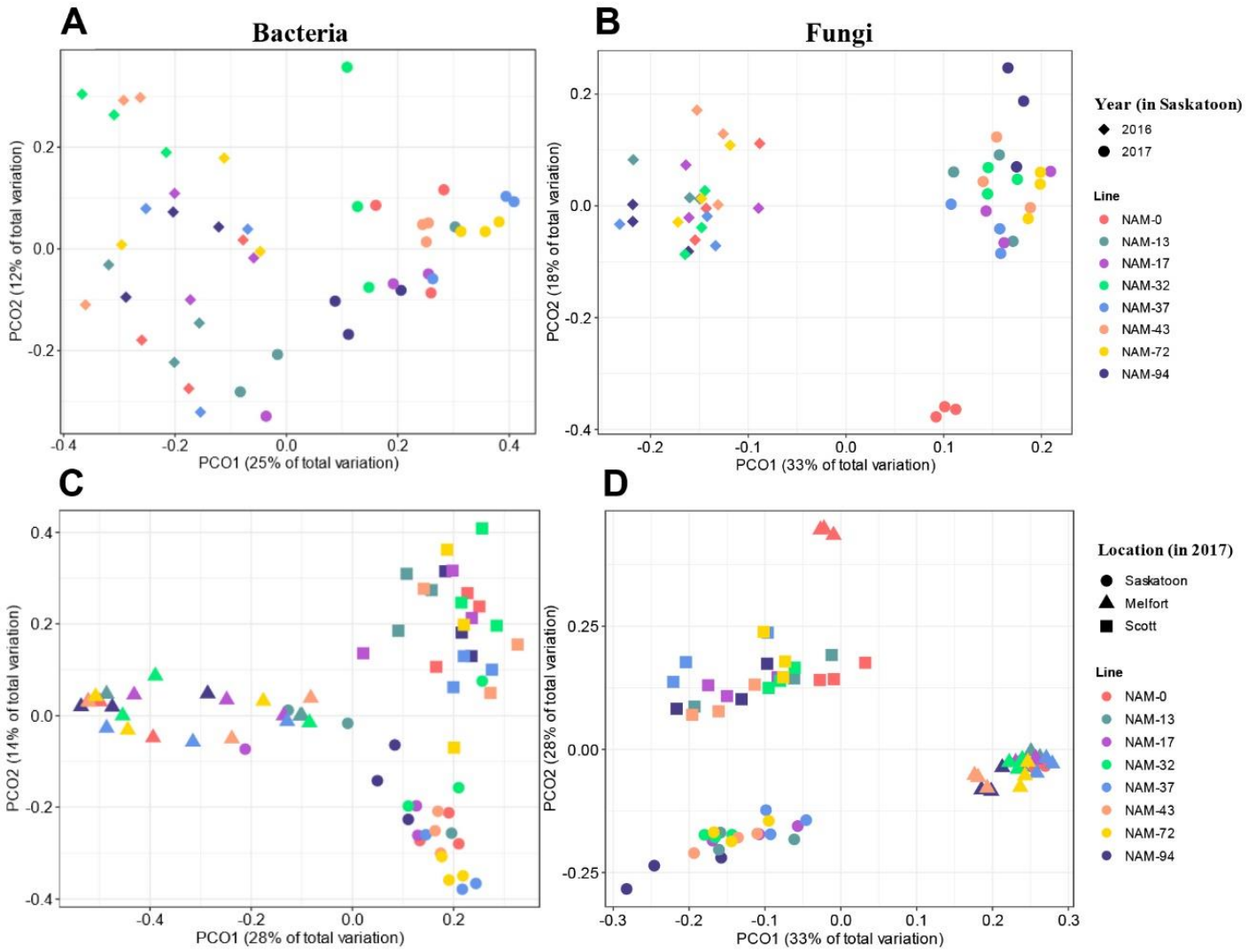


Figure 4.2 Principal coordinate analysis (PCoA) based on Bray-Curtis index of bacterial and fungal amplicon sequence variants (ASVs) from *Brassica napus* seeds harvested across two years **(A, B)** and three locations (**C, D)** in Saskatchewan. NAM= nested association mapping population.

Table 4.3 Permutational multivariate analysis of the microbial communities associated with *Brassica napus* lines.

	Bacteria			Fungi		
	F	R^2	*p*	*F*	R^2	*p*
Saskatoon(2016/2107)						
Year	11.63	0.20	0.001	21.30	0.32	0.001
Line	1.22	0.18	0.093	1.30	0.19	0.102
Year × Line	1.23	0.13	0.076	2.52	0.18	0.001
2017 (all sites)						
Location	18.00	0.34	0.001	25.87	0.43	0.001
Line	0.95	0.09	0.607	1.33	0.13	0.081
Location × Line	1.13	0.14	0.172	4.86	0.26	0.001
Saskatoon 2016						
Line	1.41	0.38	0.012	1.64	0.42	0.001
Saskatoon 2017						
Line	1.45	0.39	0.003	3.95	0.63	0.001
Melfort 2017						
Line	1.04	0.31	0.414	11.52	0.83	0.001
Scott 2017						
Line	1.29	0.36	0.055	2.05	0.47	0.001

Relative abundance of the predominant bacterial and fungal orders (> 1%) detected in the *B. napus* seed microbiome fluctuated between 2016 and 2017 in Saskatoon and among locations in 2017 (Figure 4.3; Tables J.1-J.8, Appendix J). However, all site years had high relative abundance of Gammaproteobacteria orders Enterobacteriales and Pseudomonadales and Dothideomycetes orders Pleosporales and Capnodiales. Differential abundance analysis using ANCOM showed that members of the Enterobacteriales order substantially increased while Xanthomonadales and Entylomatales substantially decreased between 2016 and 2017 at the Saskatoon location (Tables J.1 and J.2, Appendix J). Among the three locations sampled in 2017, Melfort showed a high relative abundance of members of the bacterial order Propionibacteriales (Tables J.3-J.5, Appendix J). Similarly, Scott exhibited a higher relative abundance of members of the fungal order Hypocreales whereas, at Melfort, the orders Tremellales and Sporidiobolales had higher relative abundance than at other locations (Tables J.6-J.8, Appendix J).

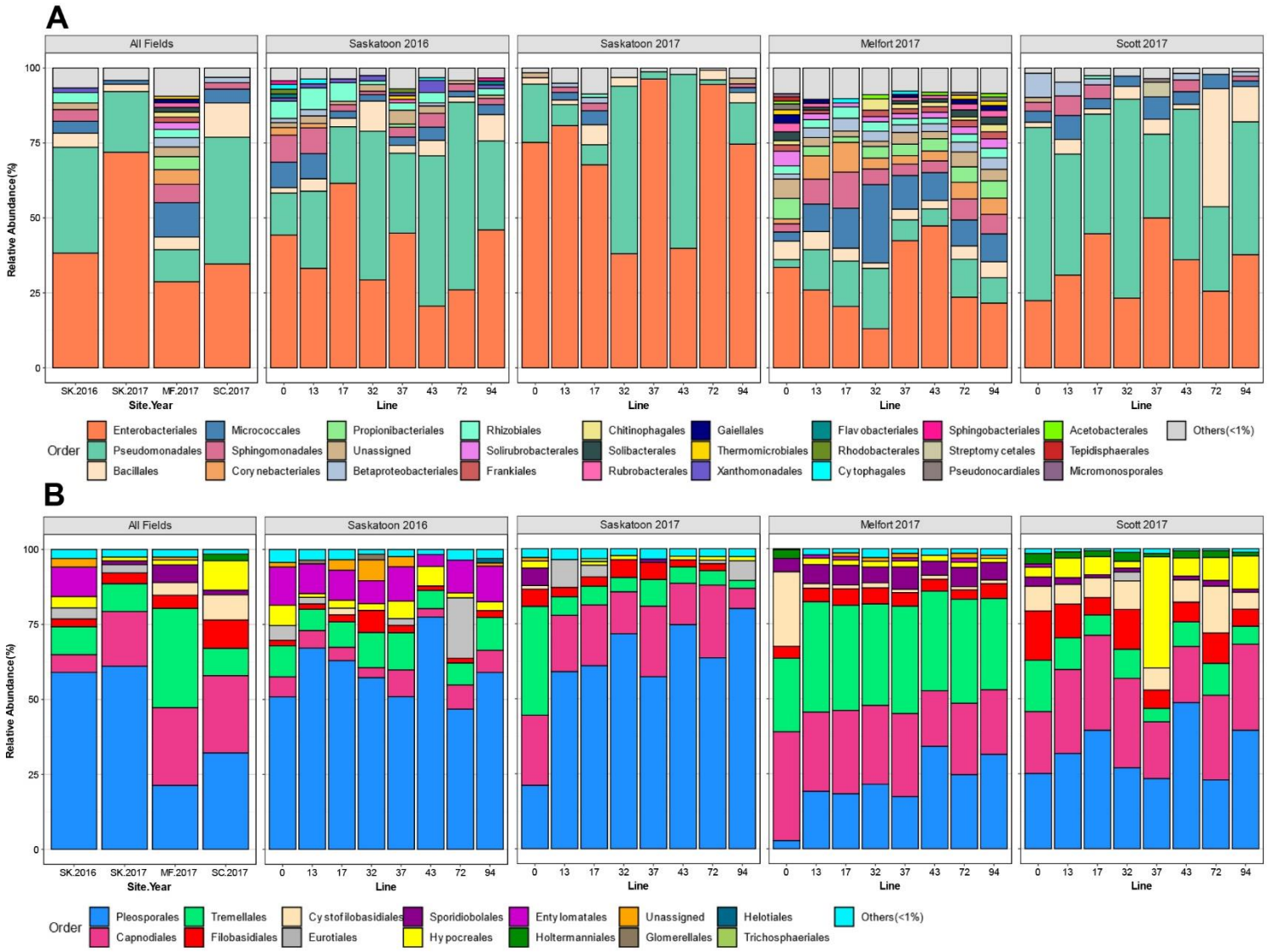


Figure 4.3 Relative abundance of the dominant bacterial **(A)** and fungal **(B)** orders in the seed microbiome of *Brassica napus* lines across years and locations. Data are shown as mean value across three replicates.

Further analysis revealed the existence of a “core microbiome” present in every replicate, line, year, and location examined; this core included 2 bacterial and 11 fungal ASVs (Table 4.2). In addition, ASVs that were present in every sample within a site also belonged to common taxonomic groups across all site years. These included members of the bacterial orders Enterobacteriales, Pseudomonadales, Micrococcales, and Sphingomonadales as well as members of the fungal orders Pleosporales, Capnodiales, Tremellales, Filobasidiales, and Sporidiobolales (Table 4.4). More specifically, *Pseudomonas* spp., *Alternaria* spp., *Chalastospora gossypii*, *Mycosphaerella tassiana*, *Vishniacozyma victoriae*, *Dioszegia hungarica*, and *Stemphylium vesicarium* were found in all site years.

Table 4.4 Taxonomic assignment of the bacterial and fungal amplicon sequence variants (ASVs) shared among all *Brassica napus* lines within a location.

BACTERIA[a]			
Saskatoon 2016 (18)	**Saskatoon 2017** (6)	**Melfort 2017** (20)	**Scott 2017** (9)
Actinobacteria (2) Bacillales-*Paenibacillus* sp. Bacillales-*Saccharibacillus* sp. Betaproteobacteriales-Burkholderiaceae Corynebacteriales-*Rhodococcus* sp. Cytophagales-*Hymenobacter* sp. (2) **Enterobacteriales** Micrococcales (2) Micrococcales-*Rathayibacter* sp. Pseudomonadales-*Pseudomonas* sp. Rhizobiales-Rhizobiaceae (2) Rhizobiales-*Methylobacterium* sp. Rhodobacterales-*Falsirhodobacter* sp. **Sphingomonadales**	Bacillales-*Exiguobacterium* sp. **Enterobacteriales** (2) Micrococcales Pseudomonadales-*Pseudomonas* sp. **Sphingomonadales**	Bacillales-*Bacillus* sp.(2) Bacillales-*Exiguobacterium* sp. Betaproteobacteriales-Burkholderiaceae (2) Corynebacteriales-*Rhodococcus* sp. Cytophagales-*Hymenobacter* sp. **Enterobacteriales** Micrococcales-Micrococcaceae (2) Micrococcales-*Rathayibacter* sp. Propionibacteriales-*Microlunatus* sp. Pseudomonadales-*Pseudomonas* sp.(4) Rhizobiales-Rhizobiaceae Rhizobiales-*Methylobacterium* sp. Rhodobacterales-*Falsirhodobacter* sp. **Sphingomonadales**	Acetobacterales Betaproteobacteriales-Burkholderiaceae Betaproteobacteriales-*Ralstonia* sp. **Enterobacteriales** Enterobacteriales-*Erwinia* sp. Micrococcales Bacillales-*Paenibacillus* sp. Pseudomonadales-*Pseudomonas* sp. **Sphingomonadales**
FUNGI[a]			
Saskatoon 2016 (32)	**Saskatoon 2017** (26)	**Melfort 2017** (19)	**Scott 2017** (30)
Capnodiales **Capnodiales-*Mycosphaerella tassiana*** (2) Entylomatales (2) Entylomatales-*Tilletiopsis washingtonensis* Filobasidiales-*Filobasidium* sp. **Filobasidiales-*Filobasidium magnum*** Hypocreales-Nectriaceae Hypocreales-*Fusarium* sp. Hypocreales-*Sarocladium strictum*	Agaricostilbales-*Kondoa sorbi* **Capnodiales** **Capnodiales-*Mycosphaerella tassiana*** (2) Entylomatales-*Tilletiopsis washingtonensis* Filobasidiales-*Filobasidium* sp. **Filobasidiales-*Filobasidium magnum*** Hypocreales-*Fusarium* sp. **Pleosporales-*Alternaria* sp**. Pleosporales-*Alternaria brassicae*	**Capnodiales** **Capnodiales-*Mycosphaerella tassiana*** (2) Cystofilobasidiales-*Cystofilobasidium macerans* (2) Dothideales-*Aureobasidium pullulans* Filobasidiales-*Filobasidium chernovii* **Filobasidiales-*Filobasidium magnum*** Holtermanniales-*Holtermanniella takashimae* **Pleosporales-*Alternaria* sp.** **Pleosporales-*Chalastospora gossypii***	**Capnodiales** **Capnodiales-*Mycosphaerella tassiana*** Cystofilobasidiales-*Cystofilobasidium macerans* (3) Filobasidiales-*Filobasidium* sp. (2) Filobasidiales-*Filobasidium chernovii* **Filobasidiales-*Filobasidium magnum*** Holtermanniales-*Holtermanniella takashimae* (2) Hypocreales-*Fusarium* sp. Hypocreales- *Trichothecium roseum* (2) Pleosporales- Didymellaceae

Table 4.4 (Continued from previous page) **FUNGI**[a]

Saskatoon 2016	Saskatoon 2017	Melfort 2017	Scott 2017
Pleosporales-*Alternaria* sp. Pleosporales-*Alternaria brassicae* Pleosporales-*Alternaria obovoidea* **Pleosporales-*Chalastospora gossypii*** (4) Pleosporales-Leptosphaeriaceae Pleosporales-*Paradendryphiella arenariae* **Pleosporales-*Stemphylium vesicarium*** Sordariomycetes Sporidiobolales-*Sporobolomyces roseus* Sporidiobolales-*Sporobolomyces ruberrimus* Tremellales-*Bulleromyces* sp. **Tremellales-*Vishniacozyma* sp.** **Tremellales-*Dioszegia hungarica*** Tremellales-*Vishniacozyma carnescens* **Tremellales-*Vishniacozyma victoriae*** (4)	**Pleosporales-*Chalastospora gossypii*** (4) Pleosporales-Leptosphaeriaceae Pleosporales-*Paradendryphiella arenariae* **Pleosporales-*Stemphylium vesicarium*** Sordariomycetes Sporidiobolales-*Sporobolomyces roseus* Tremellales-*Bulleromyces* sp. **Tremellales-*Dioszegia hungarica*** **Tremellales-*Vishniacozyma* sp.** **Tremellales-*Vishniacozyma victoriae*** (3) Tremellales-*Vishniacozyma carnescens*	**Pleosporales-*Stemphylium vesicarium*** Sporidiobolales- *Rhodotorula* sp. Sporidiobolales-*Sporobolomyces ruberrimus* **Tremellales-*Dioszegia hungarica*** **Tremellales-*Vishniacozyma* sp**. **Tremellales-*Vishniacozyma victoriae*** (3)	**Pleosporales-*Alternaria* sp.** Pleosporales-*Alternaria brassicae* Pleosporales-*Alternaria subcucurbitae* **Pleosporales-*Chalastospora gossypii*** (2) Pleosporales-*Paradendryphiella arenariae* **Pleosporales-*Stemphylium vesicarium*** Sporidiobolales- *Rhodotorula* sp. Sporidiobolales-*Sporobolomyces roseus* Tremellales-*Bulleromyces* sp. **Tremellales-*Dioszegia hungarica*** **Tremellales-*Vishniacozyma* sp.** **Tremellales-*Vishniacozyma victoriae*** (3)

[a]Taxa in bold denote members of the "core microbiome" found across locations.

4.6. Discussion

Environmental and genetic factors have been reported to shape the roots and rhizosphere microbiomes (Edwards et al. 2014; Liu et al. 2019; Simonin et al. 2020); however, their effect on other plant organs is poorly understood. Environmental conditions differ across sites and years as realized through contrasts in climate or weather as well as differences in soil type and resulting fertility. We analyzed the contribution of environment and genotype (i.e., line) in the *B. napus* seed-associated microbiota assemblage. Bacterial and fungal communities associated with eight *B. napus* lines were assessed in four different site years, two years in Saskatoon (2016, 2017) and two additional locations in 2017 (Melfort and Scott). We confirmed that environment (i.e., site year) significantly affected bacterial and fungal community assemblages, reflecting that environmental conditions including biotic or abiotic components and weather predominantly determine the seed microbiome. For example, precipitation was greater in 2016 than 2017 at the Saskatoon site, which may explain the differences in the seed microbial community structure, including richness and diversity indices (Allard et al. 2020; Mavrodi et al. 2018). Year-to-year differences in the seed microbiome was previously described in winter oilseed rape (Rochefort et al. 2019) and radish (Rezki et al. 2018). In both studies, fungi were more affected by differences between years than bacteria, supporting our findings in spring *B. napus*.

Geographical location is known to shape the microbial composition of different plant organs in several crops (Barnett et al. 2015; Gdanetz and Trail 2017; Walters et al. 2018; Kim et al. 2018), including seeds (Klaedtke et al. 2016; Walitang et al. 2018). Studies with bean (*Phaseolus vulgaris* L) (Klaedtke et al. 2016) and barley (*Hordeum vulgare*) (Chen et al. 2016) confirmed that there is a site-specific effect linked to human practices and environmental conditions, that determines microbial load and diversity in the seed microbiome. In the study carried out with barley seeds, Chen et al. (2016) reported that harvesting method and harvesting date had a significant impact on the fungal community composition. Similar to our study, this work was carried out in Western Canada, including sites in Saskatchewan.

Although the direct impact of soil type, pH, or organic matter on the seed microbiota has not been described in the literature, findings in below- and aboveground microbiomes could give us an insight into how these properties influence seeds. Previous reports suggest the presence of a communication network within plant organs, in which spermosphere, rhizosphere, and phyllosphere communities interact (Lemanceau et al. 2017; Hassani et al. 2018). Thus, microbial

shifts in one organ will be reflected in the whole phytobiome. Soil type is characterized by different physicochemical properties that, together with root exudates, mediate the formation of unique and localized microenvironments and consequently microbial community structures (Berg and Smalla 2009; Lloyd et al. 2016). Experiments carried out in the Canadian Prairies by Lay et al. (2018) and Cordero et al. (2020) showed the effect of location and soil type on bacterial communities in canola roots and rhizosphere. Our study of the seed microbiome included fields in Dark Brown (Saskatoon, Scott) and Black (Melfort) soil zones that differed not only in soil texture but pH and organic matter content. These parameters could indirectly affect seed microbial assembly by causing differences in the soil microbiome which can act as an inoculum source for the seed (Nelson, 2018). Among all soil characteristics, pH was documented in the literature as the key factor for bacterial community composition and diversity, being involved in numerous biogeochemical processes and, consequently altering bacterial growth (Hartman et al. 2008; Lauber et al. 2009). Trials with maize (*Zea mays* L.) (Tan et al. 2020), soybean (*Glycine max*) (Zhang et al. 2018), wheat (*Triticum aestivum*) (Fan et al. 2018 ; Simonin et al. 2020), and canola (Cordero et al. 2020) showed that pH regulates microbial assemblage in rhizospheric and bulk soils. In our seed study, the Scott site is characterized by low pH (approximately 5). In contrast, Melfort is not only more alkaline (pH 6.4) than Scott but has much higher organic matter content (8.2% versus 5.8%), which may have contributed to the highest richness and diversity levels found in both bacterial and fungal communities at Melfort. The correlation between organic matter content and bacterial load in roots and aerial tissues was reported before by Haron et al. (2019), who indicated that variations in soil organic matter result in different plant-associated microbial communities.

Host genotype effect on the phytobiome assemblage has been examined before in oilseed crops including soybean (Liu et al. 2019; Zhong et al. 2019), sunflower (*Helianthus annuus*) (Leff et al. 2017), olive (*Olea europaea* L.) (Mina et al. 2020), and canola (Taye et al. 2020); however, only two studies considered the effect of the host genotype on the *B. napus* seed microbiome. Endophytic and epiphytic studies with winter rapeseed revealed a cultivar-specific effect for both bacterial and fungal seed microbiomes (Rybakova et al. 2017; Rochefort et al. 2019). When canola fields were assessed in our study, a higher plant line effect was observed in fungi than in bacteria. Our results are supported by a seed microbiome analysis carried out in different plants of the Brassicaceae family (Barret et al. 2015), where the host primarily affected the fungal community composition. In addition, the geographical location of the production region highly impacts fungal

assemblage (Barret et al. 2016). The lack of seed microbiome studies considering fungi impedes a deeper analysis of the host impact. According to Andreo-Jimenez et al. (2019) an active recruitment of fungal species by the plant host can occur to cope with or as a result of stress conditions; in some cases fungi colonizes the plant to escape environmental stress.

Our results demonstrated the presence of microbial taxa shared between all lines and fields examined. *Pseudomonas* spp. as well as members of the Sphingomonadales order, previously reported as part of the core seed microbiome in *B. napus* (Links et al. 2014; Rochefort et al. 2019) were among the bacterial taxa found in every single sample in our work. In wheat cultivars, *Pseudomonas* spp. were detected in several stages of plant development, from seeds to leaf formation, suggesting that they play a critical role in plant fitness due to their ability to move systemically throughout the plant (Kuźniar et al. 2020a). Furthermore, *Pseudomonas* and *Sphingomonas* strains isolated from rice (*Oryza sativa*) seeds exhibited plant growth promotion traits, including siderophore production (Walitang et al. 2017; Ruiza et al. 2011). The *Dioszegia hungarica* fungus was also part of the core taxa. This Basidiomycota has been described in rice seeds (Eyre et al. 2019), wheat seeds (Nicolaisen et al. 2014), and wheat leaves (Ricks and Koide 2019) and is reported to be involved in suppression of plant pathogens (Hassani et al. 2018). *Alternaria* spp., *C. gossypii, M. tassiana*, and *S. vesicarium*, described in the literature as plant pathogens, were also identified here as part of the core seed microbiome. *Alternaria* spp. are known for causing dark spot disease on *Brassica* leaves (Kumar et al. 2014). Moreover, members of this genus are associated with spoilage and mycotoxin production (Bhat and Reddy 2017). Further investigation into relationships among organisms (e.g., between bacteria and fungi) can be explored to determine whether there are potential antagonistic interactions occurring that benefit plant health. For instance, Links et al. (2014) found *Pantoea* species in *Triticum aestivum* seeds able to suppress *Alternaria* spp.

B. napus seeds mainly contained members of the Enterobacteriales, Pseudomonadales, and Pleosporales bacterial orders (Links et al. 2014; Barret et al. 2015). Although, Betaproteobacteriales has been reported as one of the major orders colonizing winter rapeseed (Rybakova et al. 2017; Rochefort et al. 2019), we did not find the same pattern on spring *B. napus*. Scott received the highest precipitation across all 2017 sites which added to a delayed harvest could explain the notable occurrence of the Hypocreales order (Paul et al. 2004). A high incidence of yeasts belonging to the Tremellales order was noticed in Melfort, possibly related to high soil

organic matter content. Tremellales are basidiomycetes, which play a key role in the decomposition of organic matter (Ottesen et al. 2016; Banerjee et al. 2019). The observed shifts in microbial community composition across years and locations are driven by environment and host genotype. These shifts may also reflect plant fitness and resilience against biotic and abiotic stresses, which directly affect yield and productivity (Singh and Trivedi 2017; Berg and Raaijmakers 2018).

Acquisition, survival, and transmission of seed-associated microorganisms are determined by the mother plant (vertical transmission) as well as by agricultural management practices, weather, and seed storage conditions (horizontal transmission) (Singh and Mathur 2004; Barret et al. 2016). Our findings determined that environment and genotype influence the seed microbiome assemblage in spring *B. napus*. However, the environment appears to play a more significant role in shaping seed-associated microbial communities. Nevertheless, the existence of a core microbial taxa implies that *B. napus* plants recruit and carry bacterial and fungal species that could interact with further generations, affecting plant fitness in novel environments. Further research should consider the impacts of plant genotype-environment interactions on the entire plant microbiome (i.e., the phytobiome), with special focus on optimization in plant breeding.

5. *LENS CULINARIS* SEED MICROBIOME: ASSEMBLAGE AND TRANSMISSION[3]

5.1. Preface

Results in Chapters 3 and 4 revealed that seed-associated microbial communities are predominantly shaped by the environment in which the plants are grown. In this chapter, the seed microbiomes of seven lentil lines grown in two soils across two generations were profiled to assess the impact of soil and genotype on the assembly of the lentil seed microbiome. In addition, seed microbiomes across generations were tested to examine bacterial transmission from the mother plant to the offspring.

5.2. Abstract

Soil is an important source of bacteria and fungi for the plant, but seeds can also provide microbial inocula through heritable or stochastic assembly. Seed-associated microbial communities can potentially interact with the host plant through multiple generations. Here, I assessed the impact of two different soil types on the seed microbiome assembly of seven lentil (*Lens culinaris*) lines under environmentally controlled conditions and examined the vertical transmission of bacterial communities from seed to seed across two generations. Bulk soil microbiomes and seed microbiomes were characterized using high-throughput amplicon sequencing of the bacterial 16S rRNA gene. My results revealed that bacterial communities in the two soils differed significantly and that bacterial communities associated with seeds were significantly impacted by line (15%) in one of the soils. Co-occurrence of amplicon sequence variants (ASVs) between generations suggests members of the genera *Cutibacterium*, *Methylobacterium*, *Sphingomonas*, *Streptococcus*, and *Tepidimonas* are transmitted and preserved in lentil lines irrespective of the soil in which they were grown. Increasing our knowledge of how microbial communities carried by seeds are assembled, transmitted, and preserved offers a

[3] *Preprint*. As in the previous two chapters: ZPMM contributed in the experimental design, sampling, laboratory analysis, processing and interpretation of data, and writing the manuscript. BLH contributed in the experimental design, interpretation of data, and manuscript revisions. JJG contributed in the experimental design, interpretation of data, and manuscript revisions.

promising way for future breeding programs to consider microbial communities when selecting for more resilient and productive cultivars.

5.3. Introduction

Seed-associated microorganisms can enhance seed germination and seedling growth (Walitang et al. 2017; Verma et al. 2019), confer protection against plant pathogens (Morella et al. 2019; Yang et al. 2020), and increase host plant tolerance to salt (Dai et al. 2020) and heavy metal (Pitzschke 2018) stress. The transmission of beneficial microbes through seeds to successive generations could play an important role in plant fitness, survival, and growth under unfavorable conditions (Shahzad et al. 2018; Li et al. 2019). Recent transgenerational studies carried out in a variety of plant species including *Crotalaria pumila* (Sánchez-López et al. 2018b), *Lolium perenne* (Tannenbaum et al. 2020), *Raphanus sativus* (Rezki et al. 2018), *Triticum turgidum* (Vujanovic et al. 2019), *Triticum aestivum* (Moreira et al. 2021a), *Brassica napus* (Moreira et al. 2021a), and *Solanum lycopersicum* L. (Bergna et al. 2018) suggest microbial communities are vertically transmitted from seed to seed, thus guaranteeing their presence in the host plant progeny. A better understanding of how microorganisms carried by seeds are transmitted and preserved across generations could lead to the development of strategies to optimize microbial inheritance, with a focus on agricultural crops and food security (Berg and Raaijmakers 2018; Rana et al. 2020).

Lentil (*Lens culinaris*) is an important crop worldwide, and a key source of nutrition in low to middle income countries as their seeds contain about 24-26% protein (Boye et al. 2010; Jarpa-Parra 2018; Warne et al. 2019). In addition to having high protein content, lentils are rich in fiber, other complex carbohydrates, essential fatty acids, and a variety of vitamins (primarily vitamin B-complex) and minerals (e.g., calcium, iron, magnesium, phosphorous, potassium, and zinc) (Singh et al. 2016; Khazaei et al. 2017). Only few studies have focused on the lentil microbiome, emphasising root and rhizosphere or the effects of lentil in crop rotation (Pramanik et al. 2020; Cordero et al. 2020; Niu et al. 2018; Hamel et al. 2018). Seed microbiome assemblage and transmission in lentils remains largely unexplored.

In previous studies (Moreira et al. 2021a, Moreira et al. 2021b), we found that the environment was a key driver of seed microbiome assemblage in field-grown agricultural crops including lentil. Here, I profiled seven lentil genotypes grown during two consecutive generations in two different soils under environmentally controlled conditions to better understand the

contribution of soil type and genotype. I hypothesized that i) soil and genotype would impact the assembly of the lentil seed microbiome and that ii) bacteria would be vertically transmitted from seed to seed across generations. To test these hypotheses, I characterized bacterial seed microbiomes of seven lentil lines harvested from both soils and generations using high-throughput amplicon sequencing of the 16S rRNA gene.

5.4. Material and methods

5.4.1. Lines and Experimental Design

Seven lentil (*Lens culinaris*) lines were grown in soils collected from two different locations during two consecutive generations under environmentally controlled conditions in 2019. Basic biochemical composition of both soils (Saskatoon, Scott; Typic Boroll) was analyzed prior to initiation of the experiment (Table 5.1). The experiment was set up in 11L pots (diameter 24 cm, height 28.5 cm, Listo Products Ltd., Surrey, Canada) containing 10kg soil-sand (Industrial Quartz, Granusil®, #4095, Boucherville, Canada) mixture at a ratio of 3:1. Soils were sieved through a 4-mm sieve prior to mixing with sand. Before seeding, fertilizer (11-52-0; Terico, Western Cooperative Fertilizer Ltd, Canada, 30kg $P_2O_5ha^{-1}$) was added to each pot, followed by a 10-day pre-incubation period with moisture maintained at 25% field capacity. Lentil lines CDC KR-1, CDC Asterix, CDC Marble, CDC QG-3, Schwarze Linse, LR-30-32, and LR-30-101 from different sources (i.e., produced in different fields) (Moreira et al. 2021a; Mirali et al. 2016; Lalany and Arcand 2020) were used in my study. Twelve seeds were planted (4 cm depth) per pot, inoculant (*Rhizobium* and *Penicillium*, TagTeam® Pea and lentil; Novozymes Biologicals Limited, Saskatoon, Canada, $4.6kgha^{-1}$) was added to the seeds, and after seven days seedlings were thinned to four plants per pot. Four replicate pots per treatment in each generation were placed in a walk-in GR178 growth chamber (Conviron, Winnipeg, Canada) equipped with F54T5/HO/835/ALTO fluorescence bulbs (Philips) and 730nm PfrSpec™ near infrared bulbs (Fluence). Soil moisture in the pots were maintained by weight at 25% field capacity during the plant development period. Plants were harvested at maturity (approximately 3 months). Pests were controlled using the Insidiosus (*Orius insidiosus*) and ABS (*Amblyseius cucumeris*) systems (Biobest, Leamington, Canada). For each generation new pots of soil were prepared from the same batch of soil-sand mix (i.e., soils were not re-used for the subsequent generation; however, the soils used to grow G1 and G2 were from the same location in the same field site).

I assigned the term “G0” to the first seeds planted in the growth chamber (i.e., seeds from field grown plants), “G1” to the first seeds harvested in the growth chamber and subsequently planted to yield the second generation, and “G2” to the seeds harvested from the second generation planted in the growth chamber (Figure 5.3).

Table 5.1 Physical and chemical soil properties.

							Available				
		----------%----------					----------($mg \cdot kg^{-1}$) ----------				
Location	**pH**	**Sand**	**Silt**	**Clay**	**OC**	**OM**	**NH_4^+**	**NO_3^-**	**SO_4^{2-}**	**PO_4^{3-}**	**K^+**
Saskatoon	6.7±0.04	10.9±0.2	40.4±0.2	48.7±0.3	3.0±0.12	5.1±0.20	6.5±0.12	77.7±1.6	22.0±0.1	46.3±0.5	741±13
Scott	4.6±0.03	33.3±0.6	50.9±0.6	15.8±0.1	2.2±0.01	3.8±0.03	2.8±0.03	60.5±0.3	9.1±0.2	28.4±0.7	298±2

OC = organic carbon, OM = organic matter.

Data are mean of three technical replicates. Analyses were carried out by ALS (Saskatoon, Canada)

5.4.2. Seed harvesting, DNA extraction, and high-throughput amplicon sequencing

Lentil pods were aseptically harvested using sterile scissors, placed in sterile Whirl-Pak® bags (Nasco, Fort Atkinson, USA) and immediately transported to the laboratory where seeds were collected aseptically by opening the pods using sterile tweezers inside a biosafety cabinet. Seed DNA extraction was carried out following the method previously described by Moreira et al. (2021a). Briefly, 5g of seeds of each replicate were immersed in 25mL buffered wash solution (20 $mmolL^{-1}$ Tris-HCl, 10 $mmolL^{-1}$ EDTA, and 0.024% Triton) and shaken (150 r/min) for 15 min at room temperature followed by DNA extraction using the DNeasy® PowerWater Kit (Qiagen, Hilden, Germany). In addition, DNA extraction from Saskatoon and Scott bulk soils (Table 5.1) was performed on 0.25 g of soil using the DNeasy® PowerSoil Kit (Qiagen, Hilden, Germany) according to manufacturer instructions. Amplicon libraries were prepared following the Illumina MiSeq System Handbook (Illumina 2013) using the primer set 342F/806R (16S rRNA) with Illumina adapters (Mori et al. 2014; Moreira et al. 2021a).

5.4.3. Bioinformatics and Statistical Analysis

Primers were removed using cutadapt v.2.1 (Martin 2011), sequences were processed in QIIME2 version 2019.10 (Bolyen et al. 2019), quality filtered, and assigned to amplicon sequence variants (ASVs) in Deblur (Amir et al. 2017). A 342F- and 806R-trained V3-V4 SILVA database version 132 (Quast et al. 2013) was used to classify bacterial ASVs. α- and β-Diversity metrics were calculated in R version 4.0 using the Phyloseq package version 1.32.0 (McMurdie and Holmes, 2013). Moreover, the car package version 3.0.8 (Fox et al. 2020) and multcomp package version 1.4.13 (Hothorn et al. 2020) were both used to test differences in α-diversity values (Moreira et al. 2021b). Principal coordinate analysis (PCoA) was conducted on Hellinger transformed data to assess the bacterial community structure distribution (β diversity) using Bray-Curtis dissimilarity. Subsequently, a linear mixed-effect model was calculated for the first two extracted principal coordinates and significant axes were followed by Tukey's post-hoc tests for comparisons between treatments. Permutational multivariate analysis (PERMANOVA) were carried out in the Vegan package version 2.5.6. (Oksanen et al. 2019) with the function adonis. Furthermore, the indicator species analysis (ISA) in the PC-ORD statistical package version 6.08 (McCune and Mefford 1999) was used to identify ASV sequences that were strongly associated with seeds harvested from each soil, line, or generation analyzed in this study (i.e., ASVs present in at least half of the samples from one group and whose relative abundance in that group reaches

at least 50%, $p < 0.05$) (Dufrene and Legendre, 1997). This approach combines information on a) the specificity and b) the fidelity of occurrence of a species in a particular group, the product of these two components is called indicator value (IV) (Dufrene and Legendre, 1997). I used a cut-off indicator value of 25 to obtain the strongest indicators of any group (Dufrene and Legendre, 1997). ASVs present in all generations were identified and Venn diagrams were used to visualize the number of ASVs that were shared between generations. Bacterial α- and β-diversity metrics of each soil were calculated in R version 4.0 using the Phyloseq package version 1.32.0. Pairwise analysis of microbiome composition (ANCOM) in QIIME2 (Mandal et al. 2015) was applied to identify bacterial taxa with differential abundance among soils. Lastly, ASVs present in soils but not in G0 seeds were identified to determine potential bacterial transmission from soil to seeds.

5.5. Results

5.5.1. Differences between Saskatoon and Scott soils

The Saskatoon and Scott soils differed in physical-chemical properties (Table 5.1) and their bacterial microbiomes (Fig. K.1 and K2, Appendix K). For example, the Scott soil was sandy and more acidic (pH approximately 5), whereas pH neutral Saskatoon soil was dominated by clays and contained higher levels of measured cations and anions (Table 5.1). Soil amplicon libraries contained 1,704 bacterial ASVs from 50,655 reads in Saskatoon soil and 1,140 bacterial ASVs from 67,624 reads in Scott soil. Bacterial diversity and richness were higher in the Saskatoon versus Scott soil (Fig. K.1, Appendix K). Similarly, PCoA plots based on Bray-Curtis dissimilarity revealed differences in bacterial community structure between the two soils (PERMANOVA: $F= 59.743$, $R^2 = 0.856$, $p < 0.001$) (Fig. K.2, Appendix K). Likewise, significant differences in the relative abundance of bacterial taxa between Saskatoon and Scott soils were observed. Pairwise analysis of microbiome composition (ANCOM) revealed that Scott soil exhibited a higher relative abundance of Chloroflexi and Firmicutes phyla, while at Saskatoon a higher relative abundance was observed for members of the bacterial class Blastocatellia (Table L.1 and Fig. L.1, Appendix L).

5.5.2. α-Diversity of bacterial communities in different generations of lentil seeds

A total of 2,155,262 reads were obtained from all seed samples analyzed, which were assigned to 4416 bacterial ASVs. α-Diversity measurements revealed that G0 seeds (i.e., seeds from field grown plants) had higher bacterial richness (Chao1 index) and diversity (Inverse Simpson's index) than G1 and G2 seeds grown under environmentally controlled conditions ($p <$

0.05) (Fig. 5.1; Table M.1, Appendix M). Furthermore, when bacterial microbiomes of lentil seeds harvested from different lines were examined in each soil individually, differences in richness and diversity were observed within lines grown in Saskatoon and Scott soils (Table M.2, Appendix M). In addition, line LR-30-101 exhibited a higher richness and diversity in G2 seed microbiomes than other lines in G2 seeds from plants grown in Scott soil (Table M.2, Appendix M).

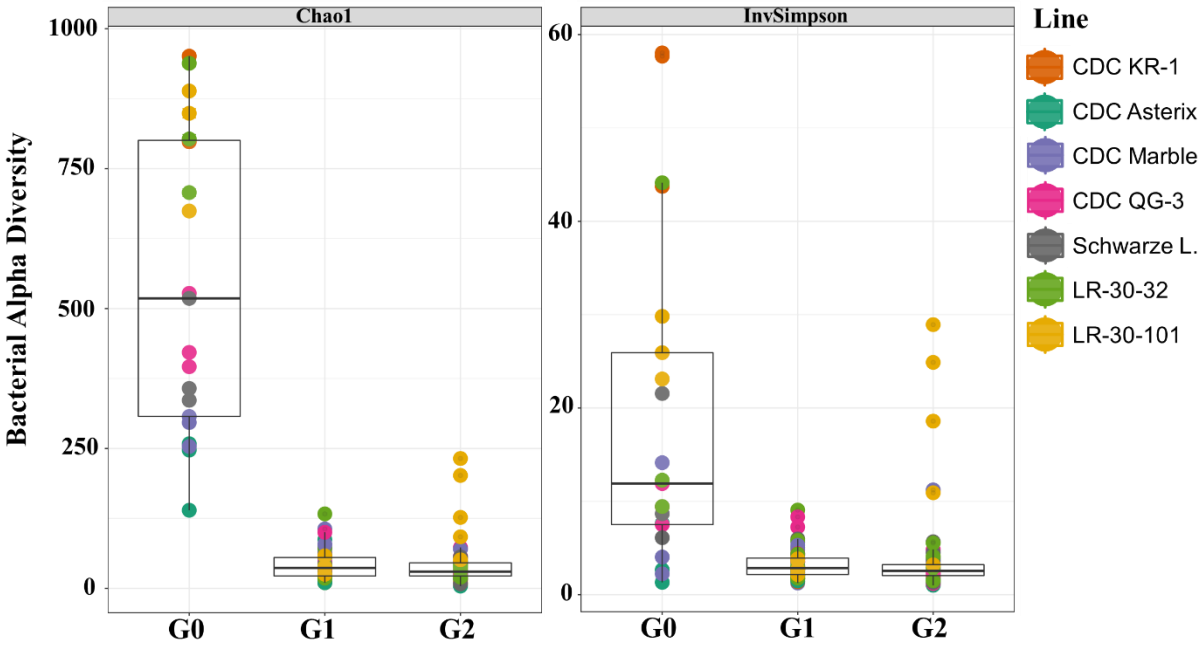


Figure 5.1 Box plots depicting Chao1 and Inverse Simpson's diversity measures of *Lens culinaris* seed bacterial communities. G0 seeds were produced in the field; G1 and G2 were produced in the growth chamber.

5.5.3. Impact of line, soil, and generation on lentil seed bacterial microbiome assemblage

Permutational multivariate analysis of variance revealed significant effects of line, soil, generation, and the interaction of generation with line on the lentil seed bacterial community composition (Table 5.2). Line and the interaction of generation with line captured the most variance (7% each, respectively), followed by soil and generation (1% each, respectively) ($p < 0.05$) (Table 5.2). When seeds harvested from different soils were analyzed separately, only bacterial communities associated with seeds grown in Scott soils were significantly impacted by line, generation, and their interaction. Specifically, for lentils grown in Scott soil, line explained up to 15% of the variance in the seed bacterial community, the interaction of generation with line explained 14% and generation alone explained 2% ($p < 0.05$) (Table 5.2; Fig. 5.2). Tukey post-hoc testing of the PCoA axes values confirmed differences between individual lines in the Scott soil (Table N.1, Appendix N).

Table 5.2 Permutational multivariate analysis of the bacterial communities associated with three generations of *Lens culinaris* lines grown under controlled conditions in Saskatoon and Scott soils.

	F	R^2	*p*
Growth Chamber Experiment			
Soil	1.762	**0.015**	**0.009**
Generation	1.552	**0.013**	**0.027**
Line	1.444	**0.073**	**0.001**
Soil × Generation	0.925	0.008	0.533
Soil × Line	1.129	0.057	0.103
Generation × Line	1.308	**0.067**	**0.004**
Soil × Generation × Line	1.048	0.053	0.263
In Saskatoon soil			
Generation	0.959	0.017	0.485
Line	1.109	0.120	0.122
Generation × Line	0.946	0.103	0.718
In Scott soil			
Generation	1.547	**0.025**	**0.027**
Line	1.483	**0.146**	**0.001**
Generation × Line	1.434	**0.141**	**0.002**

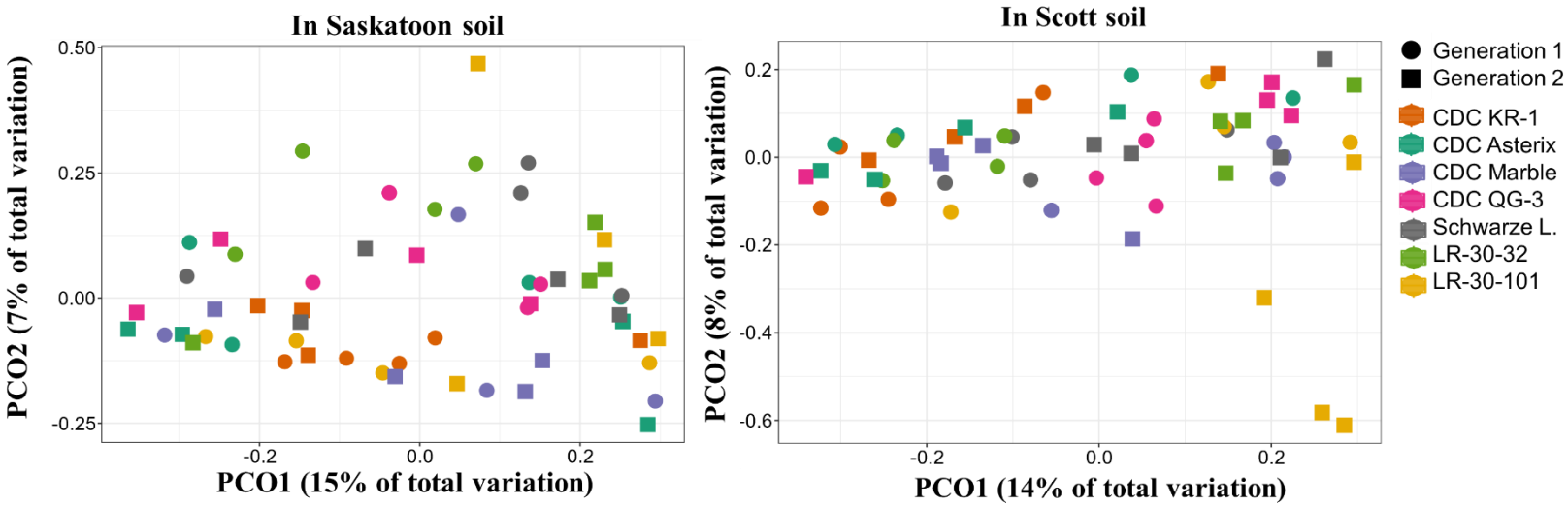


Figure 5.2 Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity index of bacterial ASVs from two generations of *Lens culinaris* seeds produced in Saskatoon and Scott soils under environmentally controlled conditions.

5.5.4. Indicator Species Analysis of bacterial communities in lentil seeds

When all seed microbiomes were analyzed together, indicator species were identified in lentil lines, generations, and samples harvested from different soils (Table 5.3). One bacterial indicator ASV was found in each of the lines CDC QG-3 (*Gaiella* sp.) and LR-30-32 (Solirubrobacterales), whereas five bacterial indicator ASVs were found in LR-30-101 (Table 5.3). In addition, indicator ASVs were associated with individual generations and also with seed samples harvested from Scott soil (*Bacillus* sp.) (Table 5.3). When indicator species analysis was performed on samples harvested from Saskatoon and Scott soils individually, a higher number of bacterial indicators were found in lines grown in Scott soil.

Table 5.3 Indicator species associated with individual lines, generation, or soil type in *Lens culinaris* seeds grown under controlled conditions in Saskatoon and Scott soils.

Group	refASV SILVA Database[a]	Lowest taxonomic Unit SILVA Database	Blast ID	Indicator Value (IV)	*p*-value
Saskatoon and Scott Soils					
Line					
CDC QG-3	2a9054ad60367a6de9e9dfa2a6531394	Gaiellales	*Gaiella* sp.	27.5	0.0014
LR-30-32	a54e44b7956420fe9eb548b46b8f5626	Solirubrobacterales	Solirubrobacterales	26.1	0.002
LR-30-101	f066fb83cc5efafacfd3717b80ce1752	*Pseudomonas* sp.	*Pseudomonas* sp.	31.0	0.0002
	38f3bc8ef8836574de0fcbeafd348b65	*Stenotrophomonas* sp.	*Stenotrophomonas maltophilia*	25.0	0.0024
	15c1252eafc2edbc8ef8da07e102d973	*Acinetobacter* sp.	*Acinetobacter calcoaceticus*	25.0	0.0024
	e40b21fce062da966d4a844eae76a1bd	*Massilia* sp.	*Massilia plicata*	25.0	0.0024
	712bf168d492f425bc7597596e8952da	Acetobacteraceae	*Roseomonas* sp.	25.0	0.003
Generation					
G1	a1bda5eb09b44cae5521c934798c7976	*Cutibacterium* sp.	*Cutibacterium* sp	44.7	0.0438
	551d23e90b0752547dc1d87355d1cd59	Gammaproteobacteria.	*Pectobacterium* sp.	26.4	0.0014
G2	8a33a7f39f3cd5ec189311cf3b500051	*Streptomyces* sp.	*Streptomyces* sp.	29.3	0.0288
Soil					
Scott	0f5c88b2e79c36e9a80b20bc80c998b1	*Bacillus* sp.	*Bacillus* sp.	34.5	0.0106
Saskatoon Soil					
Line					
CDC KR-1	2f7e079964212debf94c5445efd80d90	Acetobacteraceae	*Roseomonas* sp.	43.4	0.0022
CDC QG-3	173b6c006c32e0a520107008dc44cf16	*Gemmatimonas* sp.	*Gemmatimonas* sp.	37.5	0.0148
	1e8ce4c5197aab70fe899291e9ffdb96	*Gaiella* sp.	Actinobacteria	36.7	0.0122
	2a9054ad60367a6de9e9dfa2a6531394	Gaiellales	*Gaiella* sp.	36.4	0.0056
	1f5025eeff0b2734a9ae6fdcb994fdc5	*Methylobacterium* sp.	*Methylobacterium* sp.	32.8	0.0172
	4cd420962855afdf0bc69bb268b846c7	Sphingomonadaceae	*Sphingomonas* sp.	30.1	0.0326
Schwarze L.	5d675a3518222fc99c8cba34feaeac81	Gaiellales	Actinobacteria	31.1	0.017
LR-30-32	a54e44b7956420fe9eb548b46b8f5626	Solirubrobacterales	Solirubrobacterales	48.1	0.0014

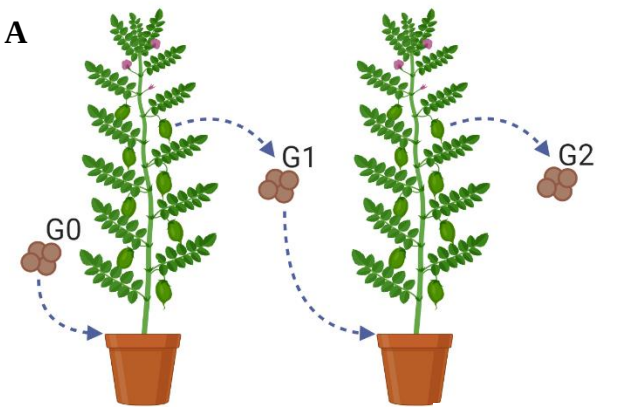
Table 5.3 (Continued from previous page)

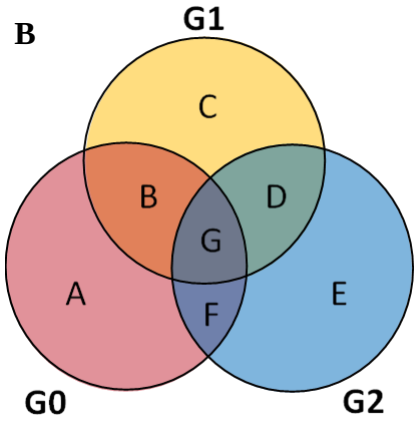
Group	refASV SILVA Database[a]	Lowest taxonomic Unit SILVA Database	Blast ID	Indicator Value (IV)	*p*-value
Generation					
G2	45107a3ca78054cd45901bb0c1d23d42	*Pseudomonas* sp.	*Pseudomonas* sp.	28.2	0.0066
Scott Soil					
Line					
CDC Marble	06cb03cddd8a12dbfaf27e8984b395c4	Sphingomonadaceae	*Sphingomonas* sp.	35.4	0.0106
	27cd48125eaac2c0bb1c2558925e292a	Enterobacteriaceae	*Pectobacterium* sp.	33.0	0.047
LR-30-101	f066fb83cc5efafacfd3717b80ce1752	*Pseudomonas* sp.	*Pseudomonas* sp.	48.8	0.001
	a6cb5951f81c2d6f6f7c303b4df53140	*Microlunatus* sp.	*Microlunatus* sp.	42.8	0.0044
	38f3bc8ef8836574de0fcbeafd348b65	*Stenotrophomonas* sp.	*Stenotrophomonas maltophilia*	37.5	0.0138
	b8040d84206511b18cae9a55e0724f21	*Bacillus* sp.	*Bacillus megaterium*	37.5	0.0138
	0a19f8ed4a31a8a54118be7fb7affbdd	*Paenibacillus* sp.	*Paenibacillus* sp.	37.5	0.0138
	e40b21fce062da966d4a844eae76a1bd	*Massilia* sp.	*Massilia plicata*	37.5	0.0138
	e5ab6dadbdcc361b84f8eb0360257ebe	*Bacillus* sp.	*Bacillus* sp.	37.5	0.0138
	712bf168d492f425bc7597596e8952da	Acetobacteraceae	*Roseomonas* sp	37.5	0.015
	15c1252eafc2edbc8ef8da07e102d973	*Acinetobacter* sp.	*Acinetobacter calcoaceticus*	37.5	0.0138
	db0bab351dd5f5efedc86bc1214bab44	*Microbacterium* sp.	*Microbacterium phyllosphaerae*	36.5	0.0138
	cf76b0f237286df02136b7575c85ca59	*Bacillus* sp.	*Bacillus megaterium*	35.6	0.0292
	a276280cde52b64a56a81dd42ed97cde	*Pseudomonas* sp.	*Pseudomonas* sp.	35.0	0.0138
	c1347ae54b0fc1dd385a1ccf3b8061ce	Sphingomonadaceae	*Novosphingobium* sp.	35.0	0.0078
	d78003f13712da48d0bb36fd45116b91	Caulobacteraceae	*Brevundimonas* sp.	27.1	0.0392
Generation					
G1	551d23e90b0752547dc1d87355d1cd59	Gammaproteobacteria	*Pectobacterium* sp.	39.3	0.0006
	7e98e06d86351bd63e51f2f098b55478	*Tepidimonas* sp.	*Tepidimonas* sp.	34.2	0.0156
	5b32100cd4e67cc6474d5bb89ee6043c	*Corynebacterium* sp.	*Corynebacterium* sp.	25.0	0.0098
G2	8a33a7f39f3cd5ec189311cf3b500051	*Streptomyces* sp.	*Streptomyces* sp.	30.8	0.0436
	2f7e079964212debf94c5445efd80d90	Acetobacteraceae	*Roseomonas* sp.	26.6	0.0142

[a]Amplicon Sequence Variants (ASVs) with significant values ($p < 0.05$) and an indicator value (IV, product of the specificity and the fidelity of occurrence of a species in a particular group) > 25 are listed (Dufrene and Legendre, 1997).

5.5.5. Vertical transmission across generations

Analysis of ASV occurrence between generations (i.e., G0, G1, and G2) suggests a small but substantial number of bacterial taxa were transmitted and preserved in all lines irrespective of the soil in which they were grown (Figure 5.3; Table O.1, Appendix O). A deeper analysis of the shared ASVs (i.e., present in all three generations), confirmed that bacterial ASVs present in G0 (i.e., seeds originating from the field) remained present in G1 and G2 seeds harvested from plants grown in both soils (Table 5.4). ASVs from *Cutibacterium*, *Methylobacterium*, *Sphingomonas*, *Streptococcus*, and *Tepidimonas* genera were not detected in the soil microbiomes, strongly suggesting that some bacteria were transmitted from one generation to the next one (i.e., from seed to seed) (Table 5.4).





	Soil	Total	A	B	C	D	E	F	G[b]
CDC KR-1	Saskatoon	1411	1246	15	47	6	56	33	**8**
	Scott	1410	1270	16	64	8	36	9	**7**
CDC Asterix	Saskatoon	590	386	27	111	8	44	10	**4**
	Scott	542	406	3	38	7	70	15	**3**
CDC Marble	Saskatoon	639	487	11	78	8	37	12	**6**
	Scott	851	463	29	212	21	102	19	**5**
CDC QG-3	Saskatoon	1016	722	16	115	15	100	33	**15**
	Scott	1010	726	26	154	6	64	24	**10**
Schwarze L.	Saskatoon	996	712	39	125	11	78	20	**11**
	Scott	990	736	22	110	13	85	19	**5**
LR-30-32	Saskatoon	1768	1379	82	164	11	80	33	**19**
	Scott	1649	1441	28	65	3	68	33	**11**
LR-30-101	Saskatoon	1727	1417	25	29	6	155	91	**4**
	Scott	1841	1349	26	83	5	216	140	**22**

[b] Numbers in bold denote ASVs present in all three generations.

Figure 5.3 Illustration of the generational study of seed bacterial microbiomes in lentil **(A)**. The seed microbiome from each generation are labeled as "G". Venn diagram depicting bacterial ASVs detected in the *Lens culinaris* seed microbiome across generations in each line **(B)**.

Table 5.4 Taxonomic assignment of the bacterial ASVs shared among all three generations in each *Lens culinaris* line harvested from plants grown in both soils and ASVs found in seeds from all generations but not in the original (bulk) soil.

Line	refASV SILVA Database [c]	Lowest taxonomic Unit SILVA Database	Blast ID
CDC KR-1	7a89fbcc332cdb3e7459401950594ae5 **a1bda5eb09b44cae5521c934798c7976** ecf7289628d49f9244469337ee1622cb 06cb03cddd8a12dbfaf27e8984b395c4 0f5c88b2e79c36e9a80b20bc80c998b1	Acetobacteraceae Actinomycetales Xanthobacteraceae Sphingomonadaceae Bacillaceae (*Bacillus* sp.)	*Gluconacetobacter* sp. *Cutibacterium* sp. *Bradyrhizobium* sp. *Sphingomonas* sp. *Bacillus* sp.
CDC Asterix	**a1bda5eb09b44cae5521c934798c7976**	Actinomycetales	*Cutibacterium* sp.
CDC Marble	**a1bda5eb09b44cae5521c934798c7976** **1f5025eeff0b2734a9ae6fdcb994fdc5**	Actinomycetales Beijerinckiaceae (*Methylobacterium* sp.)	*Cutibacterium* sp. *Methylobacterium* sp.
CDC QG-3	7a89fbcc332cdb3e7459401950594ae5 **a1bda5eb09b44cae5521c934798c7976** 06cb03cddd8a12dbfaf27e8984b395c4 0f5c88b2e79c36e9a80b20bc80c998b1 c1d9940419420113ce3fbfacc8d703a 85ec1a32516bbc535fb234ad6cb49628	Acetobacteraceae Actinomycetales Sphingomonadaceae Bacillaceae (*Bacillus* sp.) Micrococcaceae Gaiellales	*Gluconacetobacter* sp. *Cutibacterium* sp. *Sphingomonas* sp. *Bacillus* sp. *Arthrobacter* sp. Actinobacteria
Schwarze L.	7a89fbcc332cdb3e7459401950594ae5 **a1bda5eb09b44cae5521c934798c7976** 0f5c88b2e79c36e9a80b20bc80c998b1	Acetobacteraceae Actinomycetales Bacillaceae (*Bacillus* sp.)	*Gluconacetobacter* sp. *Cutibacterium* sp. *Bacillus* sp.
LR-30-32	7a89fbcc332cdb3e7459401950594ae5 **a1bda5eb09b44cae5521c934798c7976** **cce70d5962057843ac2fbadc53741e55** **82104585f0b2617c13eb69f0bbdf7d5a**	Acetobacteraceae Actinomycetales Sphingomonadaceae Streptococcaceae (*Streptococcus* sp.)	*Gluconacetobacter* sp. *Cutibacterium* sp. *Sphingomonas* sp. *Streptococcus* s
LR-30-101	**7e98e06d86351bd63e51f2f098b55478**	Burkholderiaceae (*Tepidimonas* sp.)	*Tepidimonas* sp.

[c] Taxa in bold denote ASVs found in the seeds but not in the soils.

5.5.6. Transmission from the soil to the seed

I analyzed the ASVs present in G1 and G2 seeds that were not present in G0 (Venn compartment D; Fig. 5.3 B) and identified seven ASVs in seeds grown in each soil type that might have been transmitted to the seeds from the soil (Table 5.5). Members of the orders Gaiellales and Acidobacteriales and members of the genera *Gemmatimonas* were taxa potentially transmitted to lentil seeds and were among those bacteria that had higher relative abundance in the corresponding soil bacterial community (ANCOM Table L.1, Appendix L). In addition, two of the ASVs found in Saskatoon soil (*Gaiella* sp., *Sphingomonas* sp.) and possibly transmitted to the seeds, were part of the bacterial indicator ASVs associated with lines grown in Saskatoon soil (Table 5.3).

Table 5.5 Taxonomic assignment of the bacterial ASVs found in Saskatoon and Scott soils and seeds harvested from G1 and G2.

Soil	refASV SILVA Database[d]	Lowest taxonomic Unit SILVA Database	Blast ID
Saskatoon[e,f]	db2e2e1484d09bcb17556b04ec894c65	Pseudomonadaceae (*Pseudomonas* sp.)	*Pseudomonas* sp.
	2a9054ad60367a6de9e9dfa2a6531394	Gaiellales	*Gaiella* sp.
	4cd420962855afdf0bc69bb268b846c7	Sphingomonadaceae	*Sphingomonas* sp.
	4d19cf1ea49a43b884362d80350cc6a4	Solirubrobacteraceae	Solirubrobacteraceae
	f4f7559e468e4783ed056e7b406a6fc3	Microtrichales	Acidobacteria
	2027d3e90ccc4003f1954322e99db6a9	Xanthomonadaceae (*Arenimonas* sp.)	*Arenimonas* sp.
	fba7413443d7888c3431929625e1344b	Gemmatimonadaceae	*Gemmatimonas* sp.
Scott[e]	**0c7eed5c292f582dd090dc92af4b5c2c**	Gaiellales	Gaiellales
	173b6c006c32e0a520107008dc44cf16	Gemmatimonadaceae	*Gemmatimonas* sp.
	0273707384afdd41e9d022b539094211	Gaiellales	Gaiellales
	cdd607f53e60c09054291baad7aa235d	Burkholderiaceae	*Paraburkholderia* sp.
	aff6f19c5f852510f6b4e960a077a2c8	Actinobacteria	*Catenulispora* sp.
	2c861683d369e4aea06b15a01f454795	Gaiellales	*Gaiella* sp.
	727fb1158f479567348fb8a5581347d5	Acidobacteriales	Acidobacteria

[d]ASVs found in one soil that were not present in the another.
[e] Taxa in bold denote ASVs with a relative abundance significantly different in that specific soil when compared with the another (see ANCOM results Table L.1, Appendix L).
[f] Underline taxa denote bacterial indicator ASVs (see ISA results Table 5.3)

5.6. Discussion

5.6.1. Differences between Saskatoon and Scott soils

Bacterial microbiomes in the Saskatoon and Scott soils used in my lentil transgenerational study differed in community composition, richness, and diversity. These observed biological differences between soils are likely related to differences in physicochemical properties including pH, soil texture, cation exchange capacity (CEC), organic matter content, phosphorus availability, and others (Ma et al. 2016; Fierer 2017). CEC (sum of negative sites on the soil phase that can bind cations) is positively correlated to increasing pH (Rengel et al. 2002). Thus, soil pH has a dominant effect on solubility and availability of ions. In acidic soils, availability of toxic cations (e.g., Al^{3+} and Fe^{2+}) increases while availability of essential cations (e.g., Ca^{2+}, Mg^{2+}, and K^{+}) decreases, which may reduce soil fertility (Ross et al. 2008; Rengel et al. 2011). Furthermore, pH is reported to be the main factor driving microbial community composition and abundance across soil types (Fierer and Jackson 2006; Lauber et al. 2008; Fierer et al. 2009; Rousk et al. 2010). Scott soil is characterized by a low pH (approximately 5), which could explain the significantly greater relative abundance of members of the phylum Firmicutes, a taxonomic group that is more common in acidic to near-neutral soils (Y.Zhang et al. 2017), and it is able to form endospores under stressful environmental conditions (Fimlaid and Shen 2015; Fajardo et al. 2019). Similarly, the low soil pH might drive the abundance of the phylum Chloroflexi, a metabolically diverse group, known for its ability to cope with harsh environments (Costello and Schmidt 2006; Adamczyk et al. 2019). In contrast, the Saskatoon soil is characterized for having a near-neutral pH, high clay and organic matter contents, and greater water holding capacity. These factors are typically associated with higher nitrogen mineralization rates and higher availability of nutrient ions (Ca^{2+}, Mg^{2+}, $K^{+,}$ NH_4^{+}, and NO_3^{-}) which lead to higher soil fertility (Cahn et al. 1994; Cambardella and Karlen 1999; Colman and Schimel 2013). Saskatoon soil appeared to favor some members of the class Blastocatellia, a subdivision of the phylum Acidobacteria (Adamczyk et al. 2019; Wan et al. 2020). The high abundance of members of the class Blastocatellia in Saskatoon soil when compared with Scott soil may be mostly linked to the organic matter content. Members of the phylum Acidobacteria are thought to be keystone taxa in agricultural soils due to their ability to decompose organic matter and effectively reduce nitrate and nitrite (Banerjee et al. 2016; Kalam et al. 2020).

5.6.2. α-Diversity of bacterial communities in different generations of lentil seeds

Cross-generational analysis of lentil microbiomes revealed higher bacterial richness and diversity in G0 compared to G1 or G2 seeds. This observation may be explained by the fact that G0 seeds were obtained from plants grown in the field and harvested using a typical small plot combine, thereby increasing the number of environmental and anthropogenic factors influencing the seed microbiome. In contrast, G1 and G2 seeds were obtained from plants grown under environmentally controlled conditions and harvested manually using sterile scissors inside a biosafety cabinet. Environment (Rochefort et al. 2019; Moreira et al. 2021b) and agricultural practices (Klaedtke et al. 2016), including harvesting (Chen et al. 2016) as well as seed storage (Singh and Mathur 2004; Barret et al. 2016) have been described as key drivers of seed-associated microbial assemblages in agricultural crops, which could explain the greater bacterial diversity observed in G0 seeds. Furthermore, pest control (*Orius insidiosus* and *Amblyseius cucumeris*) used in the growth chamber experiment might have influenced differences observed in G1 and G2 seed microbiomes when compared to G0. Previous reports show some insects are microbial vectors able to transmit bacterial and fungal communities to the flowers and consequently to the seeds (Rodríguez et al. 2020; Prado et al. 2020).

5.6.3. Impact of line, soil, and generation on lentil seed bacterial microbiome assemblage

When the impact of line and soil was explored in lentil seeds grown under environmentally controlled conditions (G1 and G2), line accounted for the largest source of variation, indicating that bacterial community composition in lentil seeds is determined to some extent by the host. However, when seeds harvested from Saskatoon and Scott soils were analyzed separately, only seeds from Scott soil were significantly impacted by line. This result is consistent with my previous study carried out with field grown lentil lines at the Saskatoon site, where I observed bacterial variance was not explained by plant line (Moreira et al. 2021a). Other studies carried out on agricultural crops including rice (*Oryza sativa*) (Eyre et al. 2019; Raj et al. 2019), wheat (*Triticum aestivum*) (Moreira et al. 2021a; Kuźniar et al. 2020b), winter rapeseed (*Brassica napus*) (Rybakova et al. 2017; Rochefort et al. 2019), canola (*Brassica napus*) (Moreira et al. 2021a; Moreira et al. 2021b), pumpkin (*Cucurbita pepo*) (Adam et al. 2018), and barley (*Hordeum vulgare*) (Yang et al. 2017) support the idea that the effect of host genotype (i.e., line, cultivar) on the seed microbiome assemblage is both crop- and environment-dependent, which could explain divergences found in samples harvested from Saskatoon and Scott soils. Fungal communities were

not analyzed in my study, but other studies indicate they are more affected by host genotype than bacterial communities (Barret et al. 2015; Dai et al. 2020; Moreira et al. 2021b).

Although the influence of the soil on the seed microbiome assemblage has not been thoroughly investigated, findings from growth chamber experiments carried out with rice (Hardoim et al. 2012), tomato (*Solanum lycopersicum* L.) (Bergna et al. 2018), perennial ryegrass (*Lolium perenne*) (Tannenbaum et al. 2020), and green bristle grass (*Setaria viridis*) (Rodríguez et al. 2020) suggest soil is an important source of bacterial communities inhabiting seeds. Furthermore, soil-borne microorganisms can potentially colonize seed tissues via vertical or horizontal transmission (Lemanceau et al. 2017). Thus, differences in the Saskatoon and Scott soil bacterial community composition could explain dissimilarities found in the microbiome of seeds harvested from these soils. Transgenerational studies with thale cress (*Arabidopsis thaliana*) (Truyens et al. 2016), radish (*Raphanus sativus*) (Rezki et al. 2018), tomato (Bergna et al. 2018), low rattlebox (*Crotalaria pumila*) (Sánchez-López et al. 2018b), green bristle grass (Rodríguez et al. 2020), and canola (Moreira et al. 2021a), suggested generation is another factor that also drives the assembly of bacterial communities, corroborating my findings in lentil. It is important to note that variability in seed microbiomes among generations is to some extent related to the environment in which the plants are grown and harvested (Rezki et al. 2018), which could explain why generation shaped seed microbiomes in Scott soil but not in Saskatoon soil. Moreover, lentil lines differed across generations, confirming that the interaction of generation with line also contributes to the assembly of seed microbiomes. Another possible explanation for this observation could be neutral processes related to ecological drift or stochastic changes (Nemergut et al. 2013), which are described as influencing the structure of seed-associated bacterial communities in agricultural crops (Rezki et al. 2018).

5.6.4. Indicator Species Analysis of bacterial communities in lentil seeds

Indicator species analysis (i.e., ASVs present in at least half of the samples from one group and that its relative abundance in that group reaches at least 50%, $p < 0.05$) identified one ASV classified as a *Bacillus* sp. associated with seeds harvested from Scott soil. The presence of this bacterial taxon may be linked to Scott soil physicochemical characteristics, which represent a more stressful environment than the Saskatoon soil. *Bacillus* genera are known for being well-adapted to a broad range of environments including those with extreme conditions, and for playing important roles in ecological functions such as nutrient acquisition, plant growth promotion,

protection from pathogens, drought tolerance, and others (Radhakrishnan et al. 2017; Hashem et al. 2019; Saxena et al. 2020). In lentils specifically, *Bacillus* sp. is reported to protect plants against Fusarium wilt (El-Hassan and Gowen 2006) and blight disease (*Alternaria* sp.), as well as to enhance plant growth by producing phytohormones and siderophores (Roy et al. 2018). Furthermore, *Bacillus* are known to frequently colonize seeds of other legumes (Dai et al. 2020; Mukherjee et al. 2020), gourds (Khalaf and Raizada 2016), cereals (Yang et al. 2020), and medicinal plants (Chen et al. 2018; Taghinasab and Jabaji 2020). Bacterial indicator species associated with line LR-30-101 included potential biocontrol agents such as *Massilia, Stenotrophomonas, Pseudomonas,* and *Acinetobacter*. Members of the *Massilia* genus, for example, were found in wheat, canola (Links et al. 2014), tomato (Bergna et al. 2018), and tobacco (*Nicotiana tabacum* L.) (Chen et al. 2020) seeds and have been associated with the control of the seed and seedling pathogen *Pythium aphanidermatum* (Ofek et al. 2012). Similarly, members of the *Acinetobacter* genus isolated from cucumber (*Echinocystis lobata*) seeds exhibited antagonistic properties toward the pathogenic fungi *Phytophthora capsici* (Khalaf and Raizada 2020b).

5.6.5. Vertical transmission across generations

ASVs assigned to the genera *Cutibacterium*, *Methylobacterium*, *Sphingomonas*, *Streptococcus*, and *Tepidimonas* were found in seed samples harvested from both soils and across all generations, but not in the Saskatoon and Scott soils, suggesting microbial communities were transferred directly from the mother plant (e.g., G0) to the offspring (e.g., G1, G2). Vertical transmission from seed to seed is known to occur in grasses (Vujanovic et al. 2019; Rodríguez et al. 2020; Tannenbaum et al. 2020), and there is recent evidence of vertical transmission in pulse crops (Malinich and Bauer 2018). Transmission of microbial communities seems to be directly linked to plant resilience, suggesting plants select and preserve microorganisms that will help them to adapt to novel environments or to cope with climate variability or pest/pathogen attack, thereby influencing plant fitness (Vujanovic and Germida 2017; Shahzad et al. 2018; Berg and Raaijmakers 2018). To date, three vertical transmission pathways are described for seeds: (1) through the plant vascular system, (2) through the stigma, and (3) by contact with other organs such as fruits and flowers (Maude 1996; Lemanceau et al. 2017). Further research is needed to determine which mechanism(s) can be used by lentils. One bacterial taxa I frequently observed in lentil seed samples was *Cutibacterium* (formerly *Propionibacterium*), a member of the Actinobacteria phylum previously observed in seeds of wheat (Kuźniar et al. 2020b; Abdullaeva et al. 2021), barley (Yang

et al. 2017), maize (*Zea mays* L.) (Liu et al. 2020), and rice (Raj et al. 2019). The role *Cutibacterium* plays in plants is unknown. *Methylobacterium* was also transmitted and preserved in lentil lines irrespective of the soil in which plants were grown. This genus has been reported as beneficial in *L. culinaris*, alleviating the effects of drought stress by increasing plant cytokinin levels (Jorge et al. 2019). Similar results were observed in potatoes (*Solanum tuberosum* L. cv. Desirée), in which *Methylobacterium* sp. 2A promoted plant growth, thereby alleviating salt stress. This strain also diminished the size of necrotic lesions and reduced chlorosis when potato plants were infected with *Phytophthora infestans* (Grossi et al. 2020). Moreover, *Sphingomonas* spp. isolated from seed samples are known for increasing plant growth through the production of indole acetic acid (IAA) and auxins (Ruiza et al. 2011).

5.6.6. Transmission from the soil to the seed

I also found evidence that suggests seed-associated microbial communities are recruited from the soil in which they are grown. Members of the Gaiellales order were recruited by lentil plants from both soils, this order is reported to be partly responsible for the suppression of *Fusarium oxysporum* (Ou et al. 2019), which implies that plants are recruiting microorganisms with potentially positive effects on plant fitness. According to Truyens et al. (2015) plants might be selecting certain microbiota to meet environment-specific host needs. Consequently, it may be possible to increase plant resilience and yields in different farming system through the recruitment of stable populations of beneficial microorganisms that can be subsequently transmitted to the next generation (Song et al. 2020).

In conclusion, seed microbiome assemblage in lentils depends on the soil in which the plants are grown as well as on the host genotype. However, the preservation of specific taxa across generations irrespective of the soil in which they are grown implies these microbes could be associated with plant adaptation and establishment. Collection of more information regarding microbial inheritance (e.g., Fluorescence in situ hybridization (FISH) using specific probes to detect vertical transmission) in agricultural crops is crucial for the implementation of new and sustainable strategies to explore the plant microbiome in plant breeding programs.

6. GENERAL DISCUSSION

Food security is one of the biggest challenges of the 21st century. Climate change, ecosystem degradation, soil erosion, water scarcity, and pests are some of the most common problems limiting our ability to produce sufficient food to feed an increasing world population. Plant-associated microorganisms (i.e., the plant microbiome) offer efficient and sustainable ways to improve crop yields, while supporting ecosystem services and minimizing negative environmental impacts (Arif et al. 2020; Gupta et al. 2021). For example, the use of plant-growth promoting rhizobacteria (PGPR), phosphate-solubilizing microorganisms (PSMs), nitrogen-fixing (NF) bacteria, and arbuscular mycorrhizal fungi (AMF) have the potential to reduce or replace the application of chemical fertilizers in agricultural fields, thereby contributing to a significant reduction of greenhouse gas emissions and global warming (Quiza et al. 2015; Bharati et al. 2021). Similarly, the replacement of chemical insecticides with biological control agents (BCAs) that target specific pests without harming beneficial pollinators or human health, is an innovative and promising alternative for crop protection (Kepler et al. 2017; Francis et al. 2020). BCAs can also replace chemical pesticides to control plant pathogens by using several different mechanisms such as antagonism (e.g., production of antibiotics, volatile compounds, or enzymes), competition (e.g., for space, C, N, or mineral source), parasitism, or the induction of systemic resistance (Syed Ab Rahman et al. 2018; Parulekar-Berde et al. 2021).

To date, most of bacteria and fungi used as biofertilizers or biopesticides in agricultural fields were isolated from the rhizosphere or roots of selected plant species, whereas the potential of microorganisms colonizing other plant organs remains largely unknown (Bhattacharyya and Jha 2012; Goswami et al. 2016; Verma 2019). Similarly, most information about plant-associated microorganisms comes from the analysis of culturable microbiota, whereas the nonculturable microbiota remain poorly understood. Over the last decade, advances in high-throughput sequencing technologies have dramatically improved our ability to identify and quantify microbial communities or individual microbes inhabiting plant tissues as well as to understand mechanisms of microbiome assembly and activities that contribute to overall plant health (Turner et al. 2013;

Lebeis 2014; Fricker et al. 2019). In recent years, numerous studies explored the plant microbiome of several crops, with a main focus on belowground microbial communities (i.e., microbiome associated with roots and rhizosphere soil) (Berendsen et al. 2012; Reinhold-Hurek et al. 2015; Fitzpatrick et al. 2018; Kumar and Dubey 2020). However, information regarding aboveground microbial communities (i.e., microbiome associated with leaves, flowers and seeds) is limited (Leveau 2019; Vannette 2020; Nelson 2018). Thus, the main objective of the current study was to characterize the seed microbiomes of three agricultural crops important for food security.

In the initial phase of my seed microbiome research, the endophytic microbiome was assessed but sequences obtained were mostly from plant plastid or mitochondria. For this reason, I decided to focus my analysis on the epiphytic microbiome. It should be noted that before doing so, I tested two different methodologies in order to suppress plant host plastid and mitochondrial 16S rRNA contamination, but without success. The first one used peptide nucleic acid (PNA) clamps (Lundberg et al. 2013), and the second used the 799F/1193R primer set (Chelius and Triplett 2001; Bodenhausen et al. 2013) that avoid the chloroplast amplification and separate the plant mitochondrial product from the bacterial 16S rRNA product. Other authors using these methods report the recovery of endophytic seed microbiomes. It is unclear why these methods did not work in my study, but it might be explained by (1) microbial abundance in the genotypes studied or (2) differences in the surface disinfection protocol. For instance, Rybakova et al. (2017) reported endophytes in winter *Brassica napus* seeds, but those seeds were surface-disinfected using only sterile distilled water which might not have fully removed all DNA from epiphytes. On the other hand, Robinson et al. (2016), were not able to recover endophytes from wheat when seeds were surface-disinfected with 70% ethanol, 1.5% active chlorine, and rinsed with sterile distilled water. To resolve these apparent discrepancies, coordinated studies by different laboratories using the same seed lots as well and 2-3 identical methodologies, including the one used here (65% ethanol, 5 min; 1.2% sodium hypochlorite solution, 5 min; rinsed with sterile distilled water) are needed to determine the source of these differences (e.g., microbial abundance or disinfection protocol).

In the first chapter of this work (Chapter 3), the seed microbiomes of five wheat (*Triticum aestivum*), canola (*Brassica napus*), and lentil (*Lens culinaris*) lines from two generations were characterized using high-throughput amplicon sequencing of the bacterial 16S ribosomal RNA and fungal internal transcribed spacer (ITS) regions. This study not only considered different crops, representing a broad variety of plant families (Poaceae, Brassicaceae, and Fabaceae), but also

provided information about seed-associated fungal communities, which have been overlooked in recent literature. In addition, this was the first time the *L. culinaris* seed microbiome was investigated. Results showed bacterial and fungal communities were highly differentiated by crop type, line, and generation suggesting genetic and environmental factors (i.e., field environmental conditions) are key drivers of seed microbiome assemblage in agricultural crops. This is important because it suggests that seed microbiomes assemble at least partly in response to specific biotic and abiotic stresses encountered by the plant. These findings are consistent with recent studies carried out in winter wheat seeds (Kuźniar et al. 2020b; Latz et al. 2021), where host genotype and environmental climate factors shaped bacterial and fungal seed microbiomes. Factors influencing composition and abundance of the canola seed microbiome have not been reported in the literature; however, seed microbiome studies in winter oilseed rape revealed an environmental effect (harvesting year) as well as a cultivar specific effect for both, bacterial and fungal communities (Rochefort et al. 2019).

In Chapter 4, I profiled eight genetically different *B. napus* lines harvested from four site years to better understand the relative contribution of environmental factors versus host genotype. Bacterial and fungal microbiomes of canola seeds harvested from one location in 2016 and 2017, and two additional locations in 2017 were characterized. My results revealed environment (i.e., all sources of variation that are not genetic such as location and harvesting year) plays a dominant role in shaping *B. napus* seed microbiomes, with more subtle contributions related to host plant genotype. This pattern was observed in both, bacterial and fungal microbiomes. Nevertheless, seed fungal microbiomes seem to be more affected by environment and host genotype than bacterial communities. Seed microbiome studies carried out in radish (*Raphanus sativus*) (Rezki et al. 2018) and several plants of the Brassicaceae family (Barret et al. 2015) also reported a higher genetic and environmental effect in fungal than in bacterial communities. Based on the literature, this observation could be explained by the strong influence of biogeography on fungal community composition and diversity, by the widespread pattern of dispersal limitation in fungi as well as by the ability fungi have to reproduce sexually and asexually (Taylor et al. 2006; Bruns 2019).

Based on the information collected in Chapter 3 and 4, which revealed environment was a critical factor influencing seed microbiome assemblage of agricultural crops grown in the field it was apparent a controlled environment study was needed. Thus, in Chapter 5, I profiled the seed microbiome of plants grown under environmentally controlled conditions to identify specific

factors shaping seed microbiomes. In this study, seed-associated bacterial communities of seven *L. culinaris* lines grown during two consecutive generations in two different soils in a growth chamber were characterized. My results revealed seed microbiome assemblage was driven by the soil in which the plants were grown, implying soil physical-chemical properties as well as soil-borne microbial communities influence the establishment and composition of the seed microbiota. Although the direct impact of soil on the seed microbiota is not clearly stated in the literature, studies carried out in below- and aboveground microbiomes suggest microbial community composition, richness, and diversity are affected by pH, soil texture, cation exchange capacity (CEC), organic matter content, phosphorus availability, and others (Ma et al. 2016; Fierer 2017). Microbial communities inhabiting soil may also influence seed microbiome assemblage, not only because microbial shifts in one organ are reflected in the whole phytobiome (Hassani et al. 2018; Lemanceau et al. 2017), but because of the microbial transmission from the soil to the seed, indicating soil is an important source of microbial communities inhabiting seeds (Bergna et al. 2018; Tannenbaum et al. 2020). In Chapter 5 I corroborated host genotype affects seed microbiome assemblage; however, its effect is crop- and environment-dependent (Adam et al. 2018; Raj et al. 2019; Rochefort et al. 2019).

In Chapter 5 I also found evidence suggesting seed-associated microbial communities were vertically transmitted from the mother plant to the offspring. Amplicon sequence variants (ASVs) assigned to the genera *Cutibacterium*, *Methylobacterium*, *Sphingomonas*, *Streptococcus*, and *Tepidimonas* were found in lentil seeds harvested from both soils and across all generations. Transmission of microbial communities seems to be one strategy plants use to cope with environmental changes, nutrient deficiencies, and other biotic and abiotic stresses (Shahzad et al. 2018; Berg and Raaijmakers 2018). For example, members of the *Methylobacterium* genera are reported as beneficial bacteria associated with *L. culinaris*, where *Methylobacterium oryzae* significantly enhances the performance of plants exposed to drought by producing the phytohormone cytokinin (Jorge et al. 2019).

Chapter 3, 4, and 5 findings suggest recruitment, transmission, and preservation of seed-associated microbiota were determined mainly by the environment and to some extent by the host. Interestingly, I detected a “core microbiome” in the multispecies study (Chapter 3) carried out in a single environment as well as a “core microbiome” in the multi-environment study (Chapter 4) carried out with a single plant species. Five bacterial and twelve fungal ASVs (i.e., exact ASV

match) persisted in every seed sample of *T. aestivum*, *B. napus*, and *L. culinaris* (i.e., in all lines, generations, and replicates) examined in Chapter 3. Some members of this core including *Sphingomonas* sp., *Pantoea agglomerans*, and *Vishniacozyma victoriae* are reported to be beneficial to their hosts. *Sphingomonas*, is usually found in multiple organs of mature wheat plants (Mahoney et al. 2017; Yi et al. 2020), and it is known for playing a protective role against plant pathogens including powdery mildew (*Blumeria graminis* f.sp. *tritici*) and Fusarium head blight (*Fusarium graminearum*) (Wachowska et al. 2013). Similarly, *P. agglomerans* found in wheat and canola seeds exhibits antagonism toward plant pathogenic *Alternaria* spp. (Links et al. 2014). *Pantoea agglomerans* species also have the potential to alleviate salt stress (Cherif-Silini et al. 2019) and promote plant growth in wheat (e.g., indole acetic acid biosynthesis, siderophore production) (Díaz Herrera et al. 2016), as well as to enhance drought stress tolerance in canola (Premachandra et al. 2020). *Vishniacozyma victoriae*, a member of the wheat phyllosphere microbiome (Rojas et al. 2020), is known for acting as a biological control agent against plant pathogens (Di Franceso and Baraldi 2020) such as *Penicillium* spp. and *Botrytis* spp. (Lutz et al. 2012; Cordero-Bueso et al. 2017).

I also found a "core microbiome" that included two bacterial and eleven fungal ASVs in *B. napus* lines harvested from four different site years (Chapter 4). Nevertheless, what caught my attention was the presence of common taxonomic groups across all site years (i.e., without necessarily being the exact ASV match). *Pseudomonas* spp., *Alternaria* spp., *Chalastospora gossypii*, *Mycosphaerella tassiana*, *V. victoriae*, *Dioszegia hungarica*, and *Stemphylium vesicarium* were found in samples collected from all site years. Thus, plants might be selecting certain microbiota to meet environment-specific host needs, and since "not everything is everywhere", the recruitment of species that not necessarily have the exact genetic match but play the same role may occur (Truyens et al. 2015). There is evidence that many taxonomic levels of bacteria and fungi show "ecological coherence", which means a specific taxon share general life strategies or traits that distinguish them from members of other taxa. This ecological coherence can be confounded by selective pressures acting on different niches, resulting in different genomes even for bacteria and fungi that share a common evolutionary history (Philippot et al. 2010; Treseder and Lennon 2015). Shared taxa found in Chapter 4 included species previously reported as beneficial such as *Pseudomonas* spp., *V. victoriae*, and *Dioszegia hungarica*, but also species reported as pathogenic such as *Alternaria* spp., *C. gossypii*, *M. tassiana*, and *S. vesicarium*.

Dioszegia hungarica is reported to be involved in suppression of plant pathogens (Hassani et al. 2018), whereas *Pseudomonas* spp. are recognized as high siderophore producers (Walitang et al. 2017).

Microorganisms naturally carried by seeds can potentially interact with a host plant at all stages of its development. My research provides information about bacterial and fungal communities that not only inhabit seeds, but are also transferred from the mother plant to the offspring. In addition, biotic and abiotic factors shaping seed microbiomes in agricultural crops were identified. Collectively, these findings represent an important step toward the advancement of sustainable breeding and agricultural strategies to utilize microbial communities carried by seeds for their potential contribution to plant fitness. Further research should consider relationships between bacterial and fungal communities (network dynamics) and metabolic interactions in the seed microbiome. Suggestions for future research are presented in Chapter 7.

7. FUTURE RESEARCH

The research presented in this thesis contributes important details to the still not fully comprehended factors shaping seed microbiomes. My results revealed seed microbiome assemblage in agricultural crops is driven mainly by the environment in which plants are grown with more subtle contributions related to host plant genotype. Even though my study demonstrated biotic and abiotic factors are responsible for shaping seed microbiomes, the inspection of specific factors such as management practices (fertilizer and pesticide application, harvesting methods), presence or absence of diseases, soil microbiome composition, soil salinity, drought, nutrient deficits, and others, is necessary to better understand the role of seed-microbe associations in plant health and productivity.

In the current study I also found evidence suggesting seed-associated microbial communities are vertically transmitted from the mother plant to the offspring. Future studies should consider the use of specific tools such as fluorescence *in situ* hybridization-confocal laser scanning microscopy (FISH-CLSM) to detect and monitor vertical transmission in agricultural crops (Wassermann et al. 2021).

Microbial taxa found in the seed microbiomes analyzed in my study are known to have beneficial effects on plant fitness; however, I did not explore functions or traits of these microorganisms. Future research should focus on the study of plant growth promoting mechanisms (e.g., nitrogen fixation, auxin, siderophore, indole acetic acid, and 1-aminocyclopropane-1-carboxylate deaminase production) (Johnston-Monje and Raizada 2011; Chowdhury et al. 2019), abiotic stress tolerance mechanisms (e.g., exopolysaccharides and cytokinin production) (Shahzad et al. 2018), or biological control mechanisms (e.g., competition, antagonism) in seed-borne microorganisms (Links et al. 2014; Morella et al. 2019).

Lastly, metabolic networks and their dynamic qualities in the seed microbiome remain largely unexplored. The identification of "hub microorganisms" (i.e., *keystone species that can exert strong direct and indirect effects on microbiome assembly and that function as mediators*

between the plant and its associated microbiome), using genome-wide association studies (GWASs) and metagenome-wide association studies, will provide an opportunity to promote the assembly of seed microbiomes that offer benefits to the plant (Trivedi et al. 2020).

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APPENDIX

APPENDIX A: Characteristics and source of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* seed samples.

Table A.1 Main Characteristics of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines.

Crop	Line	Main Characteristics
T. aestivum	AAC Penhold	Canadian hard red spring wheat cultivar developed at the Swift Current Research and Development Centre (SCRDC), Agriculture and Agri-Food Canada (AAFC), Swift Current, SK in 2004. This cultivar shows high yield potential and improved protein content. Resistant to leaf rust and common bunt and moderate resistant to *Fusarium* head blight and stem rust (Cuthbert et al. 2017) (Canadian Food Inspection Agency, 2019).
	AC Barrie	Canadian hard red spring wheat cultivar developed at the SCRDC, AAFC, Swift Current, SK in 1994. It combines high grain yield with high protein content, resistant to leaf and stem rust, common bunt, and loose smut (Mccaig et al.1995).
	Frontana	Brazilian wheat cultivar released in 1940, widely used *Fusarium* head blight resistant source (Steiner et al. 2004).
	Red fife	Red spring wheat cultivar with excellent bread-making quality. Originated in Galicia region of central Europe and released in Canada in 1845 (Fu et al. 2005; Mccallum et al. 2008).
	Sumai 3	Chinese spring wheat cultivar released in 1970 developed by Suzhou Institute of Agricultural Sciences in Jiangsu province. This cultivar is recognized as one of the best sources of *Fusarium* head blight resistance in the world (Zhu et al. 2019).
[a]***B. napus***	NAM 0	Canadian Line, black seed, low fiber, low erucic acid, low glucosinolate content.
	NAM 13	German cultivar (Campino), black seed, high fiber, low erucic acid, low glucosinolate content.
	NAM 17	Canadian Line, black seed, low fiber, low erucic acid, low glucosinolate content.
	NAM 37	Australian cultivar (Wesroona), black seed, high fiber, low erucic acid, high glucosinolate content.
	NAM 72	Canadian Line, yellow seed, very low fiber, low erucic acid, low glucosinolate content
[b]***L. culinaris***	CDC KR-1	Large red cultivar developed by the Crop Development Centre (CDC) at the University of Saskatchewan, Canada and released in 2009. Exhibits gray seed coat and red cotyledon colour, moderate resistant to Ascochyta blight and Anthracnose Race 1.
	CDC Asterix	Extra small green cultivar developed by the CDC at the University of Saskatchewan, Canada and released in 2012. Exhibits green seed coat and yellow cotyledon colour, moderate resistant to Ascochyta blight and intermediate resistant to Anthracnose Race 1.
	CDC Marble	French green cultivar developed by the CDC at the University of Saskatchewan, Canada and released in 2012. Exhibits green marble seed coat and yellow cotyledon colour, moderate resistant to Ascochyta blight and intermediate resistant to Anthracnose Race 1.
	CDC QG-3	Green cotyledon cultivar developed by the CDC at the University of Saskatchewan and Saskatchewan Pulse Growers, Canada and released in 2014. Exhibits green seed coat and green cotyledon colour, intermediate resistant to Ascochyta blight and moderate resistant to Anthracnose Race 1. CDC QG-3 is an imidazolinone tolerant variety.
	Schwarze Linse	Black German cultivar developed in 1964. Exhibits black seed coat and red cotyledon colour (Shaikh et al. 2013).

[a] Nested association mapping (NAM) population (Clarke et al. 2016; Mason et al. 2017; Taye et al. 2020)
[b] Government of Saskatchewan (2019), Canadian Food Inspection Agency (2019).

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Table A.2 *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* seed source.

Crop	Line	Harvest year	Field site	GPS coordinates
Parents				
T. aestivum	AAC Penhold	2015	Saskatoon, Canada	52°8'23.29"N, 106°36'49.589"W
	AC Barrie	2014	Saskatoon, Canada	52°8'49.11"N, 106°32'47.738"W
	Frontana	-	Swift Current, Canada	50°16'51.56"N, 107°45'28.63"W
	Red fife	2014	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97"W
	Sumai 3	-	Swift Current, Canada	50°16'51.56"N, 107°45'28.63"W
B. napus	NAM 0	2014, 2015	Mini-Cage Increase SK, Canada	52°8'1.568"N, 106°38'6.961"W
	NAM 13	2014	Hoop Tent SK, Canada	52°8'1.568"N, 106°38'6.961"W
	NAM 17	2014	Hoop Tent SK, Canada	52°8'1.568"N, 106°38'6.961"W
	NAM 37	2014	Hoop Tent SK, Canada	52°8'1.568"N, 106°38'6.961"W
	NAM 72	2014	Hoop Tent SK, Canada	52°8'1.568"N, 106°38'6.961"W
L. culinaris	CDC KR-1	-	Regina, Canada (Pulse Trading company)	50°27'3.013"N, 104°29'50.96"W
	CDC Asterix	2011	Saskatoon, Canada	52°9'44.56"N, 106°32'35.50"W
	CDC Marble	2012	Saskatoon, Canada	52°9'31.29"N, 106°32'35.89"W
	CDC QG-3	2012	Saskatoon, Canada	52°3'44.71"N, 106°24'47.45"W
	Schwarze Linse	2015	Saskatoon, Canada	52°3'53.86"N, 106°26'21.631"W
Offspring				
T. aestivum	AAC Penhold	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	AC Barrie	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	Frontana	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	Red fife	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	Sumai 3	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
B. napus	NAM 0	2016	Saskatoon, Canada	52°10'52.918"N, 106°30'10.587" W
	NAM 13	2016	Saskatoon, Canada	52°10'52.918"N, 106°30'10.587" W
	NAM 17	2016	Saskatoon, Canada	52°10'52.918"N, 106°30'10.587" W
	NAM 37	2016	Saskatoon, Canada	52°10'52.918"N, 106°30'10.587" W
	NAM 72	2016	Saskatoon, Canada	52°10'52.918"N, 106°30'10.587" W
L. culinaris	CDC KR-1	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	CDC Asterix	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	CDC Marble	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	CDC QG-3	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	Schwarze Linse	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W

-Exact year not determined (Anytime from 2010-2015)

APPENDIX B: α-Diversity estimators of seed-associated microbial communities in *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines across two generations.

Table B.1 α-Diversity estimators of seed-associated microbial communities in *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines across two generations.

			Chao 1*		Inverse Simpson's*	
	Crop	**Line**	**Parents**	**Offspring**	**Parents**	**Offspring**
Bacteria						
	T. aestivum	AAC Penhold	141.1 **a**	122.9 **b**	11.5 **aA**	4.8 **aB**
		AC Barrie	107.4 **bc**	129.5 **ab**	8.9 **ab**	3.7 **ab**
		Frontana	94.7 **cdB**	162.8 **aA**	9.5 **abA**	3.7 **abB**
		Red fife	124.8 **abB**	160.7 **aA**	4.8 **bc**	3 **ab**
		Sumai 3	77.2 **d**	96.9 **b**	2.5 **c**	1.4 **b**
	B. napus	NAM 0	257.0	266.6	8.8	12.0
		NAM 13	166.0	214.5	10.3	11.6
		NAM 17	298.9	179.8	9.6	8.5
		NAM 37	196.3	316.9	8.0	6.8
		NAM 72	172.2	163.2	7.8	3.3
	L. culinaris	CDC KR-1	826.2 **aA**	246.1 **B**	52.8 **aA**	9.9 **B**
		CDC Asterix	212.1**c**	203.2	3.9 **b**	16.7
		CDC Marble	282.1 **bc**	207.7	6.8 **b**	15.3
		CDC QG-3	432.7 **bA**	253.0 **B**	9.6 **b**	16.9
		Schwarze Linse	398.5 **bcA**	205.4 **B**	12.1 **b**	18.0
Fungi						
	T. aestivum	AAC Penhold	101.7 **ab**	97.0	8.4 **ab**	8.3
		AC Barrie	111.0 **ab**	109.0	7.6 **b**	10.3
		Frontana	97.3 **abB**	127.7 **A**	10.5 **a**	12.1
		Red fife	119.1 **a**	108.3	11.0 **a**	8.7
		Sumai 3	76.7 **bB**	115.7 **A**	6.8 **b**	10.1
	B. napus	NAM 0	114.4	111.7	8.3 **bc**	10.4
		NAM 13	117.3	109.3	7.6 **c**	7.2
		NAM 17	103.7	105.0	13.9 **aA**	7.4 **B**
		NAM 37	120.7	103.7	9.6 **b**	11.0
		NAM 72	116.7	91.3	9.0 **bc**	7.7
	L. culinaris	CDC KR-1	139.3 **A**	74.0 **bB**	7.5 **abA**	4.7 **B**
		CDC Asterix	105.0	75.0 **b**	6.1 **b**	6.2
		CDC Marble	113.3	90.7 **ab**	8.7 **a**	7.4
		CDC QG-3	86.3	87.7 **ab**	3.4 **c**	5.0
		Schwarze Linse	118.3	107.7 **a**	6.1 **b**	6.8

*Numbers followed by different lowercase and uppercase letter in the same columns and row, respectively, are significantly different ($p < 0.05$) as determined by Tukey post-hoc pairwise comparisons. Lowercase letters represent differences among lines within a crop and generation. Uppercase letters represent differences between generations of the same line.

APPENDIX C: Members of the bacterial and fungal "core microbiome" found in parents and offspring of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines.

Table C.1 Members of the bacterial "core microbiome" found in parents and offspring of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines.

	Bacterial Core Taxonomy		
	Parents	**Offspring**	**Parent+Offspring**
All crops	1. Enterobacteriaceae 2. Microbacteriaceae 3. Sphingomonadaceae 4. Nocardiaceae (*Rhodococcus* sp.) 5. Microbacteriaceae (*Rathayibacter* sp.)	1. Enterobacteriaceae 2. Microbacteriaceae 3. Sphingomonadaceae 4. Nocardiaceae (*Rhodococcus* sp.) 5. Microbacteriaceae (*Rathayibacter* sp.) 6. Paenibacillaceae (*Paenibacillus* sp.) 7. Burkholderiaceae 8. Pseudomonadaceae (*Pseudomonas* sp.) 9. Hymenobacteraceae (*Hymenobacter* sp.) 10. Beijerinckiaceae (*Methylobacterium* sp.) 11. Rhizobiaceae 12. Paenibacillaceae	1. Enterobacteriaceae 2. Microbacteriaceae 3. Sphingomonadaceae 4. Nocardiaceae (*Rhodococcus* sp.) 5. Microbacteriaceae (*Rathayibacter* sp.)
T. aestivum	1. Enterobacteriaceae 2. Enterobacteriaceae 3. Microbacteriaceae 4. Enterobacteriaceae 5. Sphingomonadaceae 6. Paenibacillaceae (*Paenibacillus* sp.) 7. Pseudomonadaceae (*Pseudomonas* sp.) 8. Nocardiaceae (*Rhodococcus* sp.) 9. Microbacteriaceae 10. Burkholderiaceae 11. Pseudomonadaceae (*Pseudomonas* sp.) 12. Hymenobacteraceae (*Hymenobacter* sp.) 13. Microbacteriaceae (*Rathayibacter*) 14. Rhizobiaceae 15. Burkholderiaceae 16. Burkholderiaceae 17. Burkholderiaceae 18. Pseudomonadaceae (*Pseudomonas* sp.) 19. Bacillales	1. Enterobacteriaceae 2. Enterobacteriaceae 3. Microbacteriaceae 4. Enterobacteriaceae 5. Sphingomonadaceae 6. Paenibacillaceae (*Paenibacillus* sp.) 7. Pseudomonadaceae (*Pseudomonas* sp.) 8. Nocardiaceae (*Rhodococcus* sp.) 9. Microbacteriaceae 10. Burkholderiaceae 11. Pseudomonadaceae (*Pseudomonas* sp.) 12. Hymenobacteraceae (*Hymenobacter* sp.) 13. Microbacteriaceae (*Rathayibacter* sp.) 14. Rhizobiaceae 15. Burkholderiaceae 16. Burkholderiaceae 17. Paenibacillaceae (*Paenibacillus* sp.) 18. Pseudomonadaceae (*Pseudomonas* sp.) 19. Hymenobacteraceae (*Hymenobacter* sp.) 20. Hymenobacteraceae (*Hymenobacter* sp.) 21. Beijerinckiaceae (*Methylobacterium* sp.) 22. Hymenobacteraceae (*Hymenobacter* sp.) 23. Burkholderiaceae (*Variovorax* sp.) 24. Hymenobacteraceae (*Hymenobacter* sp.) 25. Paenibacillaceae (*Saccharibacillus* sp.) 26. Actinobacteria 27. Pseudomonadaceae (*Pseudomonas* sp.)	1. Enterobacteriaceae 2. Enterobacteriaceae 3. Microbacteriaceae 4. Enterobacteriaceae 5. Sphingomonadaceae 6. Paenibacillaceae (*Paenibacillus* sp.) 7. Pseudomonadaceae (*Pseudomonas* sp.) 8. Nocardiaceae (*Rhodococcus* sp.) 9. Microbacteriaceae 10. Burkholderiaceae 11. Pseudomonadaceae (*Pseudomonas* sp.) 12. Hymenobacteraceae (*Hymenobacter* sp.) 13. Microbacteriaceae (*Rathayibacter* sp.) 14. Rhizobiaceae 15. Burkholderiaceae 16. Burkholderiaceae

Table C.1 cont. Members of the bacterial "core microbiome" found in parents and offspring of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris.*

	Bacterial Core Taxonomy		
	Parents	**Offspring**	**Parent+Offspring**
T. aestivum		28. Acetobacteraceae (*Roseomonas* sp.) 29. Weeksellaceae (*Chryseobacterium* sp.) 30. Myxococcales 31. Burkholderiaceae (*Variovorax* sp.) 32. Burkholderiaceae (*Massilia* sp.) 33. Burkholderiaceae 34. Myxococcales	
B. napus	1. Enterobacteriaceae 2. Microbacteriaceae 3. Sphingomonadaceae 4. Nocardiaceae (*Rhodococcus* sp.) 5. Burkholderiaceae 6. Pseudomonadaceae (*Pseudomonas* sp.) 7. Micrococcales 8. Hymenobacteraceae (*Hymenobacter* sp.) 9. Microbacteriaceae (*Rathayibacter* sp.) 10. Hymenobacteraceae (*Hymenobacter* sp.) 11. Beijerinckiaceae (*Methylobacterium* sp.) 12. Rhizobiaceae 13. Actinobacteria 14. Actinobacteria 15. Rhizobiaceae 16. Sphingobacteriaceae (*Pedobacter* sp.) 17. Bacillaceae (*Exiguobacterium* sp.) 18. Burkholderiaceae 19. Burkholderiaceae 20. Bacillaceae (*Bacillus* sp.) 21. Rhodobacteraceae (*Paracoccus* sp.) 22. Burkholderiaceae (*Massilia* sp.) 23. Burkholderiaceae (*Variovorax* sp.) 24. Rhodobacteraceae (*Falsirhodobacter* sp.) 25. Burkholderiaceae 26. Devosiaceae 27. Caulobacteraceae 28. Microbacteriaceae *(Microbacterium* sp.) 29. Weeksellaceae (*Chryseobacterium* sp.) 30. Enterobacteriaceae (*Buchnera* sp.)	1. Enterobacteriaceae 2. Microbacteriaceae 3. Sphingomonadacea 4. Nocardiaceae (*Rhodococcus* sp.) 5. Burkholderiaceae 6. Pseudomonadaceae (*Pseudomonas* sp.) 7. Micrococcales 8. Hymenobacteraceae (*Hymenobacter* sp.) 9. Microbacteriaceae (*Rathayibacter* sp.) 10. Hymenobacteraceae (*Hymenobacter* sp.) 11. Beijerinckiaceae (*Methylobacterium* sp.) 12. Rhizobiaceae 13. Actinobacteria 14. Actinobacteria 15. Rhizobiaceae 16. Sphingobacteriaceae (*Pedobacter* sp.) 17. Paenibacillaceae (*Paenibacillus* sp.) 18. Paenibacillaceae (*Saccharibacillus* sp.) 19. Microbacteriaceae (*Rathayibacter* sp.) 20. Microbacteriaceae 21. Rhodobacteraceae (*Falsirhodobacter* sp.) 22. Enterobacteriaceae (*Rosenbergiella* sp.) 23. Myxococcales	1. Enterobacteriaceae 2. Microbacteriaceae 3. Sphingomonadaceae 4. Nocardiaceae (*Rhodococcus* sp.) 5. Burkholderiaceae 6. Pseudomonadaceae (*Pseudomonas* sp.) 7. Micrococcales 8. Hymenobacteraceae (*Hymenobacter* sp.) 9. Microbacteriaceae (*Rathayibacter* sp.) 10. Hymenobacteraceae (*Hymenobacter* sp.) 11. Beijerinckiaceae (*Methylobacterium* sp.) 12. Rhizobiaceae 13. Actinobacteria 14. Actinobacteria 15. Rhizobiaceae 16. Sphingobacteriaceae (*Pedobacter* sp.)
L. culinaris	1. Enterobacteriaceae 2. Microbacteriaceae 3. Enterobacteriaceae 4. Sphingomonadaceae	1. Enterobacteriaceae 2. Microbacteriaceae 3. Enterobacteriaceae 4. Sphingomonadaceae	1. Enterobacteriaceae 2. Microbacteriaceae 3. Enterobacteriaceae 4. Sphingomonadaceae

Table C.1 cont. Members of the bacterial "core microbiome" found in parents and offspring of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines.

	Bacterial Core Taxonomy		
	Parents	**Offspring**	**Parent+Offspring**
L. culinaris	5. Nocardiaceae (*Rhodococcus* sp.) 6. Microbacteriaceae (*Rathayibacter*) 7. Bacillaceae (*Bacillus* sp.) 8. Beijerinckiaceae (*Methylobacterium* sp.) 9. Micrococcaceae 10. Azospirillaceae (*Skermanella* sp.) 11. Pseudomonadaceae (*Pseudomonas* sp.)	5. Nocardiaceae (*Rhodococcus* sp.) 6. Microbacteriaceae (*Rathayibacter* sp.) 7. Bacillaceae (*Bacillus* sp.) 8. Beijerinckiaceae (*Methylobacterium* sp.) 9. Micrococcaceae 10. Rhizobiaceae 11. Actinobacteria 12. Paenibacillaceae (*Saccharibacillus* sp.) 13. Pseudomonadaceae (*Pseudomonas* sp.) 14. Enterobacteriaceae 15. Pseudomonadaceae (*Pseudomonas* sp.) 16. Microbacteriaceae (*Rathayibacter* sp.) 17. Microbacteriaceae (*Microbacterium* sp.) 18. Actinobacteria 19. Sphingomonadaceae 20. Actinobacteria 21. Sphingomonadaceae (*Sphingomonas* sp.) 22. Rhizobiaceae 23. Rhizobiaceae (*Aureimonas* sp.) 24. Caulobacteraceae 25. Rhodobacteraceae (*Falsirhodobacter* sp.) 26. Rhizobiaceae 27. Sphingobacteriaceae (*Pedobacter* sp.) 28. Weeksellaceae (*Chryseobacterium* sp.) 29. Hymenobacteraceae (*Hymenobacter* sp.) 30. Paenibacillaceae (*Paenibacillus* sp.) 31. Pseudomonadaceae (*Pseudomonas* sp.) 32. Pseudomonadaceae (*Pseudomonas* sp.) 33. Burkholderiaceae 34. Paenibacillaceae (*Paenibacillus* sp.) 35. Burkholderiaceae 36. Pseudomonadaceae (*Pseudomonas* sp.) 37. Micrococcales	5. Nocardiaceae (*Rhodococcus* sp.) 6. Microbacteriaceae (*Rathayibacter* sp.) 7. Bacillaceae (*Bacillus* sp.) 8. Beijerinckiaceae (*Methylobacterium* sp.) 9. Micrococcaceae

Table C.2 Members of the fungal "core microbiome" found in parents and offspring of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines.

	Fungal Core Taxonomy		
	Parents	**Offspring**	**Parent+Offspring**
All crops	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Fusarium* sp. 8. *Filobasidium* sp. 9. *Vishniacozyma* sp. 10. *Filobasidium magnum* 11. *Dioszegia hungarica* 12. *Mycosphaerella tassiana* 13. *Cystofilobasidium macerans*	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Fusarium* sp. 8. *Filobasidium* sp. 9. *Vishniacozyma* sp. 10. *Filobasidium magnum* 11. *Dioszegia hungarica* 12. *Mycosphaerella tassiana* 13. *Cladosporium delicatulum* 14. *Bulleromyces* sp. 15. *Tilletiopsis washingtonensis* 16. *Sporobolomyces ruberrimus* 17. Pleosporaceae 18. *Sporobolomyces roseus* 19. *Vishniacozyma victoriae* 20. *Vishniacozyma carnescens*	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Fusarium* sp. 8. *Filobasidium* sp. 9. *Vishniacozyma* sp. 10. *Filobasidium magnum* 11. *Dioszegia hungarica* 12. *Mycosphaerella tassiana*
T. aestivum	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Fusarium* sp. 8. *Filobasidium* sp. 9. *Vishniacozyma* sp. 10. *Filobasidium magnum* 11. *Sporobolomyces roseus* 12. *Dioszegia hungarica* 13. *Cystofilobasidium macerans* 14. *Chalastospora gossypii* 15. *Parastagonospora poae* 16. *Mycosphaerella tassiana* 17. *Vishniacozyma carnescens* 18. *Chalastospora gossypii* 19. *Chalastospora gossypii* 20. *Parastagonospora poae* 21. *Vishniacozyma victoriae* 22. *Vishniacozyma carnescens* 23. *Sarocladium strictum* 24. *Pyrenophora* sp. 25. *Aureobasidium pullulans* 26. Dothideomycetes	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Fusarium* sp. 8. *Filobasidium* sp. 9. *Vishniacozyma* sp. 10. *Filobasidium magnum* 11. *Sporobolomyces roseus* 12. *Dioszegia hungarica* 13. *Cystofilobasidium macerans* 14. *Chalastospora gossypii* 15. *Parastagonospora poae* 16. *Mycosphaerella tassiana* 17. *Vishniacozyma carnescens* 18. *Chalastospora gossypii* 19. *Chalastospora gossypii* 20. *Parastagonospora poae* 21. *Vishniacozyma victoriae* 22. *Vishniacozyma carnescens* 23. *Dioszegia* sp. 24. Phaeosphaeriaceae 25. *Zymoseptoria brevis* 26. *Cladosporium delicatulum* 27. *Fusarium* sp. 28. *Bulleromyces* sp. 29. *Tilletiopsis washingtonensis* 30. *Sporobolomyces ruberrimus* 31. Xylariales 32. *Vishniacozyma victoriae* 33. *Vishniacozyma carnescens* 34. *Parastagonospora poae* 35. *Parastagonospora* sp. 36. *Filobasidium chernovii* 37. *Papiliotrema* sp. 38. *Vishniacozyma* sp. 39. *Bipolaris* sp.	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Fusarium* sp. 8. *Filobasidium* sp. 9. *Vishniacozyma* sp. 10. *Filobasidium magnum* 11. *Sporobolomyces roseus* 12. *Dioszegia hungarica* 13. *Cystofilobasidium macerans* 14. *Chalastospora gossypii* 15. *Parastagonospora poae* 16. *Mycosphaerella tassiana* 17. *Vishniacozyma carnescens* 18. *Chalastospora gossypii* 19. *Chalastospora gossypii* 20. *Parastagonospora poae* 21. *Vishniacozyma victoriae* 22. *Vishniacozyma carnescens*

Table C.2 cont. Members of the fungal "core microbiome" found in parents and offspring of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines.

	Fungal Core Taxonomy		
	Parents	**Offspring**	**Parent+Offspring**
T. aestivum		40. *Vishniacozyma victoriae* 41. Pleosporaceae 42. Unassigned	
B. napus	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Cladosporium delicatulum* 8. *Alternaria brassicae* 9. Leptosphaeriaceae 10. *Fusarium* sp. 11. *Fusarium* sp. 12. *Stemphylium vesicarium* 13. *Bulleromyces* sp. 14. *Tilletiopsis washingtonensis* 15. *Filobasidium* sp. 16. *Vishniacozyma* sp. 17. *Filobasidium magnum* 18. *Sporobolomyces ruberrimus* 19. Pleosporaceae 20. *Sporobolomyces roseus* 21. *Dioszegia hungarica* 22. Entylomatales 23. *Vishniacozyma victoriae* 24. *Chalastospora gossypii* 25. *Vishniacozyma carnescens* 26. Entylomatales 27. *Sarocladium strictum* 28. *Filobasidium* sp. 29. *Alternaria oudemansii* 30. *Mycosphaerella tassiana* 31. *Vishniacozyma carnescens* 32. *Rhodotorula* sp. 33. *Sarocladium summerbellii* 34. *Acremonium rutilum* 35. *Sclerotinia sclerotiorum* 36. *Cystofilobasidium macerans* 37. *Vishniacozyma tephrensis*	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Cladosporium delicatulum* 8. *Alternaria brassicae* 9. Leptosphaeriaceae 10. *Fusarium* sp. 11. *Fusarium* sp. 12. *Stemphylium vesicarium* 13. *Bulleromyces* sp. 14. *Tilletiopsis washingtonensis* 15. *Filobasidium* sp. 16. *Vishniacozyma* sp. 17. *Filobasidium magnum* 18. *Sporobolomyces ruberrimus* 19. Pleosporaceae 20. *Sporobolomyces roseus* 21. *Dioszegia hungarica* 22. Entylomatales 23. *Vishniacozyma victoriae* 24. *Chalastospora gossypii* 25. *Vishniacozyma carnescens* 26. Entylomatales 27. *Sarocladium strictum* 28. *Filobasidium* sp. 29. *Alternaria oudemansii* 30. *Mycosphaerella tassiana* 31. *Chalastospora gossypii* 32. *Holtermanniella takashimae* 33. *Chalastospora gossypii* 34. *Hannaella coprosmae* 35. Sordariomycetes	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Cladosporium delicatulum* 8. *Alternaria brassicae* 9. Leptosphaeriaceae 10. *Fusarium* sp. 11. *Fusarium* sp. 12. *Stemphylium vesicarium* 13. *Bulleromyces* sp. 14. *Tilletiopsis washingtonensis* 15. *Filobasidium* sp. 16. *Vishniacozyma* sp. 17. *Filobasidium magnum* 18. *Sporobolomyces ruberrimus* 19. Pleosporaceae 20. *Sporobolomyces roseus* 21. *Dioszegia hungarica* 22. Entylomatales 23. *Vishniacozyma victoriae* 24. *Chalastospora gossypii* 25. *Vishniacozyma carnescens* 26. Entylomatales 27. *Sarocladium strictum* 28. *Filobasidium* sp. 29. *Alternaria oudemansii* 30. *Mycosphaerella tassiana*
L. culinaris	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Cladosporium delicatulum* 8. *Fusarium* sp. 9. *Stemphylium vesicarium* 10. *Bulleromyces* sp. 11. *Filobasidium* sp. 12. *Vishniacozyma* sp. 13. *Filobasidium magnum* 14. Pleosporaceae 15. Sclerotiniaceae 16. *Dioszegia hungarica* 17. *Mycosphaerella tassiana*	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Cladosporium delicatulum* 8. *Fusarium* sp. 9. *Stemphylium vesicarium* 10. *Bulleromyces* sp. 11. *Filobasidium* sp. 12. *Vishniacozyma* sp. 13. *Filobasidium magnum* 14. Pleosporaceae 15. Sclerotiniaceae 16. *Dioszegia hungarica* 17. *Mycosphaerella tassiana* 18. *Hannaella coprosmae*	1. *Alternaria* sp. 2. *Mycosphaerella tassiana* 3. *Chalastospora gossypii* 4. *Vishniacozyma victoriae* 5. *Vishniacozyma victoriae* 6. *Vishniacozyma victoriae* 7. *Cladosporium delicatulum* 8. *Fusarium* sp. 9. *Stemphylium vesicarium* 10. *Bulleromyces* sp. 11. *Filobasidium* sp. 12. *Vishniacozyma* sp. 13. *Filobasidium magnum* 14. Pleosporaceae 15. Sclerotiniaceae 16. *Dioszegia hungarica* 17. *Mycosphaerella tassiana*

Table C.2 cont. Members of the fungal "core microbiome" found in parents and offspring of *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* lines.

	Fungal Core Taxonomy		
	Parents	**Offspring**	**Parent+Offspring**
L. culinaris	18. *Aspergillus proliferans* 19. *Cystofilobasidium macerans* 20. *Filobasidium chernovii*	19. *Fusarium* sp. 20. *Sclerotinia sclerotiorum* 21. *Tilletiopsis washingtonensis* 22. *Sporobolomyces ruberrimus* 23. *Sporobolomyces roseus* 24. *Colletotrichum lentis* 25. *Vishniacozyma victoriae* 26. *Vishniacozyma carnescens* 27. Sordariomycetes 28. *Papiliotrema* sp.	

APPENDIX D: Differentially abundant bacterial and fungal ASVs identified by ANCOM when comparing the seed microbiomes of three agricultural crops across two generations.

Table D.1 Differentially abundant bacterial ASVs identified by ANCOM when comparing *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* seed microbiomes across two generations.

ASV	**W	*Reject null	WheatOffspring					WheatParent					
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
4ff2ce294e6225baec6bd00cb57ad074	568	TRUE	1	1	1	1	5	3	18	90	128.5	216	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae
22d9aed4d524b13b749abc7b1e515486	537	TRUE	5	16	23	30.5	72	1	1	1	1	5	D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Myxococcales

ASV	**W	*Reject null	CanolaOffspring					CanolaParent					
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
99b3f0f472a923d6a905a01f0d836e2b	1601	TRUE	1	1	1	7	29	38	120	267	587.5	2004	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Buchnera
f85e0ad6ec1269099703d64c81c3366e	1592	TRUE	1	1	1	2	11	1	44	71	124	286	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Buchnera
d8dd0705dd011456cefd4e3ac9a840e6	1579	TRUE	6	26	37	76	358	1	1	2	4	9	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;D_5__Falsirhodobacter
327d4d6ce0e897b5da757c92ef0f4e00	1570	TRUE	1	47	54	88	359	1	1	1	1	50	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae
64eb2494dbf4892b1fcfc8aae01b7158	1554	TRUE	1	10	27	43	376	1	1	1	1	11	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingomonas
2d8695f45cac585649776080c6e2a55b	1545	TRUE	10	33	55	82.5	486	1	1	1	7.5	84	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Paenibacillus
37d881e695f49ebf8804166cd64de4f9	1544	TRUE	1	1	2	5	8	4	16.5	34	44.5	82	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae
674330d2712cf4dece0dee9c4fa4902b	1514	TRUE	1	1	644	2052	11513	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Erwinia
adb640879aa9d7a4d4e2014f3bf2980d	1512	TRUE	5	9	15	32.5	44	58	78	95	134.5	254	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae
a1bda5eb09b44cae5521c934798c7976	1491	TRUE	1	1	1	1	4	1	4	8	10.5	38	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Propionibacteriales;D_4__Propionibacteriaceae;D_5__Cutibacterium
68258ac111ba68754c4fd1eee40ac5de	1490	TRUE	1	1	3	5	28	8	14	22	45.5	65	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Massilia

ASV	**W	*Reject null	LentilOffspring					LentilParent					
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
72d751b53f43eae22dd0fbfdb6091c63	2349	TRUE	43	102	248	307	1907	1	1	1	2	13	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
10f50071098e6cca71f48e435706a1d5	2345	TRUE	2	47.5	159	267.5	386	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria
591650397089e03dfe873be0226b5532	2328	TRUE	10	85	224	350	494	1	1.5	3	5.5	14	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Saccharibacillus
ef3465f9c9d52bff054d1df730929339	2327	TRUE	1	78	141	289.5	624	1	1	1	1	7	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
df07796a0f0c32103e4889e16c38952d	2317	TRUE	8	59	104	292.5	1206	1	1	1	2	357	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
09c8e6dc871954c9b30dedff9a46e89e	2310	TRUE	11	24.5	51	90.5	222	1	1	1	3	6	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria
5a68a2ced1175ce230b78cd9cb462648	2305	TRUE	4	20	29	38.5	65	1	1	1	1	2	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Rhizobiaceae
64eb2494dbf4892b1fcfc8aae01b7158	2303	TRUE	6	19	25	30.5	46	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingomonas
c20acdc310e52797cb206883b3eba505	2296	TRUE	1	28.5	57	99.5	353	1	1	1	2.5	5	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Saccharibacillus
22fba850115dd415bc0556cdef6dc577	2294	TRUE	1	12	30	50.5	163	1	1	1	1	2	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Rhizobiaceae;D_5__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
98bcb1b74afd32517170c3da64daf523	2293	TRUE	24	91	220	385	1668	1	1	3	12.5	149	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Paenibacillus
d8dd0705dd011456cefd4e3ac9a840e6	2288	TRUE	9	14	35	70.5	117	1	1	1	3	11	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;D_5__Falsirhodobacter
55f47e06fb9580066754a0fb66fbea5a	2285	TRUE	16	49.5	61	93.5	213	1	1	1	2.5	79	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Microbacterium
d9182f5d2767987ad66dea978c37cda4	2284	TRUE	1	10.5	30	45.5	139	1	1	1	1	3	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria
f066fb83cc5efafacfd3717b80ce1752	2277	TRUE	59	128	225	488.5	1873	1	4.5	10	22	288	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
fbb68742aac7d6e4dc1589359e99f1f5	2265	TRUE	6	12.5	39	71.5	113	1	1	1	4.5	14	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Weeksellaceae;D_5__Chryseobacterium
0ad7e95e50c7d8bb1892bbd3fd6f0737	2260	TRUE	1	6.5	42	77.5	793	1	1	1	1	1	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Saccharibacillus
531efdc4c2102566dbec467775e4da8f	2260	TRUE	4	12.5	18	33	52	1	1	1	2	8	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Rhizobiaceae
a78502abc40f13bb7549bd4be6ab45c2	2259	TRUE	1	12.5	49	105.5	133	1	1	1	1	12	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
28e2c2e581b768b883d582c50e87e885	2251	TRUE	1	7.5	15	19.5	43	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Xanthomonadales;D_4__Xanthomonadaceae;D_5__Stenotrophomonas
2d8695f45cac585649776080c6e2a55b	2247	TRUE	51	104.5	349	697	4667	1	6.5	12	45.5	235	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Paenibacillus
296e5aef73b121c1d811c0e8732231eb	2245	TRUE	1	4.5	9	32.5	82	1	1	1	1	3	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__Flavobacterium
73fed31595dbfab1585b1f7affb05498	2222	TRUE	11	22	40	69	90	1	2.5	6	9.5	16	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales
545f313453aaeef5c994971a2c8f7148	2220	TRUE	5	8.5	25	31.5	107	1	1	3	4.5	9	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__Pedobacter
f14f8d4035339bd7ced048bc8b8dbeab	2213	TRUE	1	6	26	75	379	1	1	1	3.5	11	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Weeksellaceae;D_5__Chryseobacterium
36b09829f7d02bd0156267d12e98b4fc	2206	TRUE	1	7.5	16	23	39	1	1	1	1	4	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae
1f5025eeff0b2734a9ae6fdcb994fdc5	2195	TRUE	1	1	1	1	1	1	8	17	23.5	41	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium
5148f7390ffbe9639a70d46a69ee599b	2156	TRUE	19	68.5	104	170	338	1	4	15	33.5	68	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Rathayibacter
80c4c4c36cb4fc185eaa36a641583edd	2148	TRUE	1	3.5	10	21.5	120	1	1	1	1	3	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Massilia
45107a3ca78054cd45901bb0c1d23d42	2134	TRUE	1	1	1	60.5	368	37	74	686	3307	14137	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

Table D.2 Differentially abundant fungal ASVs identified by ANCOM when comparing *Triticum aestivum*, *Brassica napus*, and *Lens culinaris* seed microbiomes across two generations.

ASV	**W	*Reject null	WheatOffspring					WheatParent					
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
1ac7001628cd5926185b585586fb058d	780	TRUE	168	527.5	931	1179	2558	1	1	1	7	96	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Rhynchogastremataceae;g__Papiliotrema
fad4e28e32b38df9f6b7216dee063980	776	TRUE	58	78.5	118	303	648	1	1	1	1	36	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Bipolaris
5e557a68e85ee03eaebc52062dcdff98	773	TRUE	721	1856.5	2341	4152	5703	1	1	7	387	1211	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Xylariales
bbd410c02034a7911f096abf70d5dfad	761	TRUE	3455	4455.5	7661	9333	10944	1	33.5	73	1211	5817	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
f6319c80505cfc39f49e0fdbb1f64b7e	760	TRUE	47	132	222	719.5	9371	1	1	1	32.5	353	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora
bfb189f6ade06c5933ccb7c6aa29495d	749	TRUE	307	576.5	910	1614.5	1835	1	35.5	60	111.5	219	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Sporobolomyces;s__Sporobolomyces_ruberrimus
4fcd2da624d73729ebcc439a541a7f0b	745	TRUE	540	819	927	1312	1549	1	35.5	74	121	424	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Tremellaceae;g__Bulleromyces;s__unidentified
f005bb8a7befe140577df369f966ef0e	725	TRUE	71	116	143	184.5	202	215	661	1168	3319.5	14945	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Filobasidiales;f__Filobasidiaceae;g__Filobasidium;s__unidentified
7bd3c3df5b7a80c46e14ad8f5e6be892	716	TRUE	1	35	53	87	167	1	1	1	1	34	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Rhodotorula
fd117529a30825ea0f76b6ca7ea0aa1e	711	TRUE	45	66	94	310	1023	1	1	1	58	77	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__unidentified

ASV	**W	*Reject null	CanolaOffspring					CanolaParent					
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
75a9b73903a3053226dff54454c05cd9	897	TRUE	1	50	133	273	871	1	1	1	1	10	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Erysiphales;f__Erysiphaceae;g__Blumeria;s__Blumeria_graminis
67f58713ac805bd9dbcd6cd68872df31	887	TRUE	1	1	1	1	1	7	25.5	52	142	464	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Rhodotorula
325b04fce747ff9791507c60555994eb	886	TRUE	526	1768.5	3828	8795	21909	22	85.5	161	226	1591	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Leptosphaeriaceae;g__unidentified;s__unidentified
569c21099e4b7d0598acc0a35d197007	878	TRUE	1	1	4	7.5	19	23	57.5	79	101	169	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Acremonium;s__Acremonium_rutilum
4c709c385717473e12b752fb44a4fd81	876	TRUE	1	1	1	2.5	12	15	20.5	32	63.5	142	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Sarocladium;s__Sarocladium_summerbellii
386e54fe292c2f421b3f407434f6d9b8	874	TRUE	1	1	117	477	934	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Glomerellales;f__Plectosphaerellaceae;g__Plectosphaerella
a7b84bdccfd4af32fcc14f18b818e6aa	860	TRUE	1	1	10	25.5	50	21	116.5	208	410.5	846	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_tephrensis
5dfdda12a5800fb11bc3ee736f2df12a	858	TRUE	1	4	22	40	95	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Leptosphaeriaceae;g__Ampelomyces;s__Ampelomyces_quisqualis
7eb776a43a89eb41525e639fdeb54b43	845	TRUE	1	4.5	28	50.5	216	1	1	1	1	4	k__Fungi;p__Ascomycota;c__Sordariomycetes
c1a139a96f5296a7f7be52a3b44e485d	845	TRUE	1	1	1	1	1	1	1	156	224.5	374	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
5a5a657103448a51240c20591cdb61b4	820	TRUE	14	31	47	59.5	122	108	179	262	465.5	851	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Sarocladium;s__Sarocladium_strictum
d0f63ae4057c8d978e554cc723b10414	812	TRUE	145	351	441	604	888	1159	1903	2379	3996	7063	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae

ASV	**W	*Reject null	LentilOffspring					LentilParent					
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
0d8f1c3f3e4425b3d40726c1edf7f937	968	TRUE	1638	3016	3759	5321.5	8493	1	1	9	50.5	302	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Glomerellales;f__Glomerellaceae;g__Colletotrichum;s__Colletotrichum_lentis
49076be858535133180b0c688159899a	967	TRUE	55	90	128	198	604	1	1	1	1	7	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
a9be25c1f5877c27b288acf18dd6e97f	967	TRUE	1	1	1	1	6	13	32.5	100	1089	16004	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Eurotiales;f__Aspergillaceae;g__Aspergillus;s__Aspergillus_proliferans
c39ddb3469eef1b997ee59c419b57d26	961	TRUE	736	2151	3452	10282.5	21719	1	1	56	120	4677	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Helotiales;f__Sclerotiniaceae;g__Sclerotinia;s__Sclerotinia_sclerotiorum
5e601f1cbc8b00d3a58e7d6d1f39baae	937	TRUE	16	132.5	220	336.5	833	1	1	10	57.5	174	k__Fungi;p__Ascomycota;c__Sordariomycetes
ebc9a61cf82bc67fe1c129fcbd96592f	927	TRUE	281	2346	3472	6998.5	21798	42	143.5	246	311	1524	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Helotiales;f__Sclerotiniaceae
1ac7001628cd5926185b585586fb058d	918	TRUE	81	124	203	296	442	1	14	30	47.5	62	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Rhynchogastremataceae;g__Papiliotrema
b0f188f5375515db6ff8bdafc2095eeb	906	TRUE	1	7	11	13.5	74	12	55	377	798.5	1781	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Cystofilobasidiaceae;g__Cystofilobasidium;s__Cystofilobasidium_macerans
82516ed0f0cfbddd67f45d1693baa15f	897	TRUE	1	8.5	13	28	103	1	1	1	1	6	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Bionectriaceae;g__Clonostachys;s__Clonostachys_miodochialis
83cbd2de93b9aa5206ed87138fc957a1	879	TRUE	35	54	93	127	364	1	5.5	15	22	150	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Hannaella;s__Hannaella_coprosmae

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

Table D.3 Differentially abundant bacterial ASVs identified by ANCOM when comparing the seed microbiomes of three agricultural crops grown in Saskatoon, Canada in 2016.

ASV	**W	*Reject null	CanolaOff					WheatOff					
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
2692a3daa302b0bc427b41db96a33ffa	1285	TRUE	1	1	1	1	2286	3746	9852.5	11979	13009.5	19089	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae
5fd2507be8d352399835cc82a000e46e	1279	TRUE	1	1	1	3.5	7	49	78	89	127.5	184	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
29f47b376cd3c406db25f8a1c97fe83c	1272	TRUE	1	1	1	1	11	8	21.5	44	73	104	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae
327d4d6ce0e897b5da757c92ef0f4e00	1260	TRUE	1	47	54	88	359	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae
307138ed09b9b3f73f5c3dff23b58568	1260	TRUE	1	1	1	1	6	1	22.5	46	94	128	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae
d8dd0705dd011456cefd4e3ac9a840e6	1258	TRUE	6	26	37	76	358	1	1	1	3.5	6	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;D_5__Falsirhodobacter
864894c4b7f6513e20f50b1f7b0a5529	1251	TRUE	1	1	1	1	1	1	11.5	15	61	132	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Massilia
ec4c1b6bdf70c2215b2a61e1447964f4	1239	TRUE	1	1	1	1	1	1	10.5	25	50	1957	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
0869444fa1adb8715cd38cc7975851b2	1239	TRUE	24	38.5	73	107	311	1	1	1	5	68	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
64eb2494dbf4892b1fcfc8aae01b7158	1239	TRUE	1	10	27	43	376	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingomonas
5b7b47abc72e62c91c178ad85c0ba921	1233	TRUE	1	1	1	1	1	2	6	12	20.5	69	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Massilia
6eb3b99fcd5bf2c06c00a8d0d2994153	1232	TRUE	1	1	1	1	1	1	6	7	16.5	44	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
b23eac12ade1116079bde73d2abbee42	1229	TRUE	1	1	4	6.5	31	9	17.5	37	71	110	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
ff07078a4c3630162107126ef7c55849	1228	TRUE	1	1	1	1	1	1	5.5	12	27.5	62	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae
e67349f0e42b34d0002caa5fb00d4eb6	1227	TRUE	1	1	1	1	8	4	9.5	12	18.5	27	D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Myxococcales
09c8e6dc871954c9b30dedff9a46e89e	1226	TRUE	9	20.5	33	79.5	283	1	1	3	3	13	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria
a78502abc40f13bb7549bd4be6ab45c2	1210	TRUE	1	6	18	39	74	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
7a83ceb382b27aaf48785d10adabc515	1204	TRUE	1	1	1	2	6	1	7.5	10	20.5	26	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
db0bab351dd5f5efedc86bc1214bab44	1199	TRUE	1	9.5	26	42	165	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Microbacterium
59b29ded280e52e101b8b00c76119122	1192	TRUE	1	1	1	7	56	4	13.5	27	39	80	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
62ca5cf890a330a3ea826d8aedbdad69	1187	TRUE	1	1	1	1	3	1	6	9	15	103	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
6f4e6f3e07346f7b98305b599389833f	1186	TRUE	3	5	18	42.5	982	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Rosenbergiella
22d9aed4d524b13b749abc7b1e515486	1177	TRUE	1	2	4	5.5	31	5	16	23	30.5	72	D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Myxococcales
fbf4f6e283aa711b3007a0d022b399fe	1174	TRUE	1	1	1	7	169	1	128.5	214	441.5	606	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingomonas
fc1d9940419420113ce3fbfacc8d703a	1166	TRUE	1	4.5	13	58	471	1	1	1	1	3	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Micrococcaceae

ASV	**W	*Reject null	LentilOff					WheatOff					
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
2692a3daa302b0bc427b41db96a33ffa	1050	TRUE	1	1	1	1	916	3746	9852.5	11979	13009.5	19089	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae
72d751b53f43eae22dd0fbfdb6091c63	1043	TRUE	43	102	248	307	1907	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
29f47b376cd3c406db25f8a1c97fe83c	1041	TRUE	1	1	1	1	1	8	21.5	44	73	104	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae
df07796a0f0c32103e4889e16c38952d	1040	TRUE	8	59	104	292.5	1206	1	1	1	1	7	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
b23eac12ade1116079bde73d2abbee42	1035	TRUE	1	1	1	2.5	5	9	17.5	37	71	110	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
ef3465f9c9d52bff054d1df730929339	1031	TRUE	1	78	141	289.5	624	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
fc1d9940419420113ce3fbfacc8d703a	1028	TRUE	7	24.5	85	114.5	296	1	1	1	1	3	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Micrococcaceae
712bf5cae78d734b4f1b29c6a4c9bf7b	1022	TRUE	1	23.5	66	290	835	1	1	1	1	3	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Serratia
307138ed09b9b3f73f5c3dff23b58568	1018	TRUE	1	1	1	1	17	1	22.5	46	94	128	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae
5a68a2ced1175ce230b78cd9cb462648	1016	TRUE	4	20	29	38.5	65	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Rhizobiaceae
864894c4b7f6513e20f50b1f7b0a5529	1015	TRUE	1	1	1	1	1	1	11.5	15	61	132	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Massilia
fbf4f6e283aa711b3007a0d022b399fe	1012	TRUE	1	1	1	1	18	1	128.5	214	441.5	606	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingomonas
64eb2494dbf4892b1fcfc8aae01b7158	1009	TRUE	6	19	25	30.5	46	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingomonas
5fd2507be8d352399835cc82a000e46e	1003	TRUE	1	3	5	15.5	61	49	78	89	127.5	184	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
e67349f0e42b34d0002caa5fb00d4eb6	1003	TRUE	1	1	1	1	3	4	9.5	12	18.5	27	D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Myxococcales
ec4c1b6bdf70c2215b2a61e1447964f4	1001	TRUE	1	1	1	1	1	1	10.5	25	50	1957	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
09c8e6dc871954c9b30dedff9a46e89e	1001	TRUE	11	24.5	51	90.5	222	1	1	3	3	13	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria
555150f7f4b6ef9a9db9861501dd51cf	998	TRUE	1	7.5	9	17.5	46	30	67.5	129	155	281	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
5b7b47abc72e62c91c178ad85c0ba921	998	TRUE	1	1	1	1	1	2	6	12	20.5	69	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Massilia
22fba850115dd415bc0556cdef6dc577	998	TRUE	1	12	30	50.5	163	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Rhizobiaceae;D_5__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
5148f7390ffbe9639a70d46a69ee599b	998	TRUE	19	68.5	104	170	338	1	2	6	9.5	19	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Rathayibacter
a78502abc40f13bb7549bd4be6ab45c2	997	TRUE	1	12.5	49	105.5	133	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
22d9aed4d524b13b749abc7b1e515486	996	TRUE	1	1	1	4	6	5	16	23	30.5	72	D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Myxococcales
327d4d6ce0e897b5da757c92ef0f4e00	991	TRUE	4	10.5	21	42.5	70	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae
d8dd0705dd011456cefd4e3ac9a840e6	990	TRUE	9	14	35	70.5	117	1	1	1	3.5	6	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;D_5__Falsirhodobacter
ff07078a4c3630162107126ef7c55849	990	TRUE	1	1	1	1	1	1	5.5	12	27.5	62	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae
56c2cc6c1619141b747f4e9afd49a0c1	990	TRUE	1	29	50	196.5	1569	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Erwinia
70a09696c923f5f9294a6eff31abf25e	990	TRUE	1	29	41	166	1193	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Erwinia
7a83ceb382b27aaf48785d10adabc515	988	TRUE	1	1	1	1	3	1	7.5	10	20.5	26	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
1f5025eeff0b2734a9ae6fdcb994fdc5	986	TRUE	1	1	1	1	1	1	5.5	7	21	60	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium
0f5c88b2e79c36e9a80b20bc80c998b1	984	TRUE	3	22	38	66.5	113	1	1	1	3	6	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Bacillaceae;D_5__Bacillus
5d51a23509ca7d3c73345edc10ad48e8	981	TRUE	12	117	292	447	5808	1	1	1	24.5	440	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae
d9182f5d2767987ad66dea978c37cda4	971	TRUE	1	10.5	30	45.5	139	1	1	1	1.5	3	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria
a510e82b88623b42b94f01b936bb7def	959	TRUE	1	2.5	4	5.5	9	1	20	32	45.5	65	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
62ca5cf890a330a3ea826d8aedbdad69	959	TRUE	1	1	1	1	3	1	6	9	15	103	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
b344487a7443d2fc200a7f55dd5fa494	951	TRUE	1	1	1	3	5	1	9.5	15	19.5	37	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
853845a1e103541839232fd473300e29	948	TRUE	1	1	1	1	1	1	3.5	5	7	21	D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Myxococcales
73b7a87c38be1977bf6cfd35ccf59d41	945	TRUE	1	1.5	4	4.5	11	4	15.5	28	40	83	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter

Table D.3 cont. Differentially abundant bacterial ASVs identified by ANCOM when comparing the seed microbiomes of three agricultural crops grown in Saskatoon, Canada in 2016.

ASV	**W	*Reject null	CanolaOff					LentilOff					
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
db2e2e1484d09bcb17556b04ec894c65	1465	TRUE	1	100.5	341	912.5	15372	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
674330d2712cf4dece0dee9c4fa4902b	1443	TRUE	1	1	644	2052	11513	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Erwinia
df07796a0f0c32103e4889e16c38952d	1441	TRUE	1	1	1	1	954	8	59	104	292.5	1206	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
3c66e2899f73859d4cd81e0407cfbe49	1435	TRUE	4	6	9	16	131	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Myxococcales
55f47e06fb9580066754a0fb66fbea5a	1431	TRUE	1	1	1	6	38	16	49.5	61	93.5	213	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Microbacterium
712bf5cae78d734b4f1b29c6a4c9bf7b	1427	TRUE	1	1	1	1	46	1	23.5	66	290	835	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Serratia
d9182f5d2767987ad66dea978c37cda4	1423	TRUE	1	1	1	1	1	1	10.5	30	45.5	139	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria
0869444fa1adb8715cd38cc7975851b2	1399	TRUE	24	38.5	73	107	311	1	1	1	19.5	71	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
5a68a2ced1175ce230b78cd9cb462648	1392	TRUE	1	1	1	1	99	4	20	29	38.5	65	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Rhizobiaceae
0ad7e95e50c7d8bb1892bbd3fd6f0737	1391	TRUE	1	1	1	1	1	1	6.5	42	77.5	793	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Saccharibacillus
6f4e6f3e07346f7b98305b59938983 3f	1364	TRUE	3	5	18	42.5	982	1	1	1	1	8	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Rosenbergiella
b78490cfccc78d71ed5a5ae3ff92bdcd	1352	TRUE	1	1	1	1	8	1	6	14	20	50	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Corynebacteriales;D_4__Nocardiaceae;D_5__Rhodococcus
296e5aef73b121c1d811c0e8732231eb	1345	TRUE	1	1	1	1	11	1	4.5	9	32.5	82	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__Flavobacterium
5148f7390ffbe9639a70d46a69ee599b	1320	TRUE	3	5	11	27	89	19	68.5	104	170	338	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Rathayibacter

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

Table D.4 Differentially abundant fungal ASVs identified by ANCOM when comparing the seed microbiomes of three agricultural crops grown in Saskatoon, Canada in 2016.

ASV	**W	*Reject null	CanolaOff					WheatOff					
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
325b04fce747ff9791507c60555994eb	866	TRUE	526	1768.5	3828	8795	21909	1	1	1	3	8	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Leptosphaeriaceae;g__unidentified;s__unidentified
1ac7001628cd5926185b585586fb058d	865	TRUE	1	1	1	1	8	168	527.5	931	1179	2558	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Rhynchogastremataceae;g__Papiliotrema
81dd4b112b3a91da16e30434fcd7ba1e	865	TRUE	2310	3654	5338	8120.5	11940	1	5	10	13.5	21	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_brassicae
5d3e3dfd137f3b94cea23f71de637b0d	864	TRUE	388	1201	1596	1758	4013	1	1	1	5	11	k__Fungi;p__Basidiomycota;c__Exobasidiomycetes;o__Entylomatales;f__unidentified;g__unidentified;s__unidentified
0673bdbc8a6e165af5af2c8689302d70	863	TRUE	168	543	725	765.5	1388	1	1	1	10	30	k__Fungi;p__Basidiomycota;c__Exobasidiomycetes;o__Entylomatales;f__unidentified;g__unidentified;s__unidentified
d9f58908e54fea3618f22844d0c556a6	861	TRUE	21	107	128	152	2598	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_oudemansii
4eac723a6a9e8217f28c2ab549602833	860	TRUE	1	1	1	1	46	242	403.5	603	992.5	1965	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
5e557a68e85ee03eaebc52062dcdff98	859	TRUE	1	2	4	15	93	721	1856.5	2341	4152	5703	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Xylariales
c39ddb3469eef1b997ee59c419b57d26	857	TRUE	1	48	92	218	658	1	1	1	1	16	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Helotiales;f__Sclerotiniaceae;g__Sclerotinia;s__Sclerotinia_sclerotiorum
8a5726aa2c7721a33c6cf40a51a920b5	857	TRUE	1	1	1	1	19	46	82	96	153	545	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
f6319c80505cfc39f49e0fdbb1f64b7e	857	TRUE	1	1	1	1	12	47	132	222	719.5	9371	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora
75a9b73903a3053226dff54454c05cd9	857	TRUE	1	50	133	273	871	1	1	1	3	17	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Erysiphales;f__Erysiphaceae;g__Blumeria;s__Blumeria_graminis
9d6eddf36e03d4d20c1ad1cdfa130688	856	TRUE	1	32	80	431.5	7068	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Trichothecium;s__Trichothecium_roseum
a9be25c1f5877c27b288acf18dd6e97f	855	TRUE	1	20.5	121	2485.5	16683	1	1	1	1	8	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Eurotiales;f__Aspergillaceae;g__Aspergillus;s__Aspergillus_proliferans
fd117529a30825ea0f76b6ca7ea0aa1e	855	TRUE	1	1	1	1	30	45	66	94	310	1023	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__unidentified
dd9a6a4f9596f0541b4001eb9d6f4fbf	854	TRUE	1	1	1	22.5	41	235	427	601	742.5	2027	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
568dbc1d0aad7dde78fe451cee3261c3	854	TRUE	1	1	1	1	38	47	128.5	165	217.5	429	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
cbbb5beb9ee83e5bcf8016569a53e433	853	TRUE	1	1	1	1	14	34	65	70	107.5	168	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae
fe07aa60f9231191add4319a41b448cb	852	TRUE	1	1	1	1	57	84	122.5	139	157	363	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
bbd410c02034a7911f096abf70d5dfad	850	TRUE	16	80	114	180.5	2227	3455	4455.5	7661	9333	10944	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
fad4e28e32b38df9f6b7216dee063980	849	TRUE	1	1	1	1	143	58	78.5	118	303	648	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Bipolaris
5e601f1cbc8b00d3a58e7d6d1f39baae	841	TRUE	27	145.5	203	417.5	2502	1	2.5	11	38.5	153	k__Fungi;p__Ascomycota;c__Sordariomycetes
84843c411ce2b93404f860cd2bcf44d7	830	TRUE	1	1	1	1	33	10	16.5	44	62.5	127	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Mycosphaerellaceae;g__Zymoseptoria;s__Zymoseptoria_brevis
386e54fe292c2f421b3f407434f6d9b8	825	TRUE	1	1	117	477	934	1	1	1	1	10	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Glomerellales;f__Plectosphaerellaceae;g__Plectosphaerella
7313efe22060402792d7042e44d3282c	824	TRUE	1	1	1	1	8	1	10.5	14	22	46	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Mrakiaceae;g__Itersonilia;s__Itersonilia_pannonica
7eb776a43a89eb41525e639fdeb54b43	824	TRUE	1	4.5	28	50.5	216	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes
5dfdda12a5800fb11bc3ee736f2df12a	821	TRUE	1	4	22	40	95	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Leptosphaeriaceae;g__Ampelomyces;s__Ampelomyces_quisqualis
d84337197873c342e9077961140b14b6	815	TRUE	1	1	1	1	1	1	18	21	33.5	190	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Rhodotorula;s__Rhodotorula_graminis
8f26708a09ff3158fcfc07e801eddf70	805	TRUE	677	1889	2637	3234	6012	184	277.5	396	544.5	949	k__Fungi;p__Basidiomycota;c__Exobasidiomycetes;o__Entylomatales;f__Entylomatales_fam_Incertae_sedis;g__Tilletiopsis;s__Tilletiopsis_washingtonensis
ced910020ea568dbe8a1b472815ffe41	799	TRUE	1	1	1	3	7	1	12.5	19	25	46	k__Fungi
8b0dfcd895be861832041188cefb49e1	793	TRUE	346	711.5	902	1174	2414	3491	5053.5	5566	6503.5	8131	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
f034ec725857a24818422228b7fe8b54	791	TRUE	27	85	131	171	1373	190	849	1074	1894	4270	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
d0f63ae4057c8d978e554cc723b10414	791	TRUE	145	351	441	604	888	983	1930	2951	4479.5	5248	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
f33b4c66388d6dac4cc2cec25f941ded	782	TRUE	1	1	7	12.5	36	24	30.5	40	48	62	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_carnescens

ASV	**W	*Reject null			LentilOff					WheatOff			
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
ebc9a61cf82bc67fe1c129fcbd96592f	790	TRUE	281	2346	3472	6998.5	21798	1	1	1	6	14	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Helotiales;f__Sclerotiniaceae
0d8f1c3f3e4425b3d40726c1edf7f937	790	TRUE	1638	3016	3759	5321.5	8493	1	1	1	1	7	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Glomerellales;f__Glomerellaceae;g__Colletotrichum;s__Colletotrichum_lentis
c39ddb3469eef1b997ee59c419b57d26	790	TRUE	736	2151	3452	10282.5	21719	1	1	1	1	16	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Helotiales;f__Sclerotiniaceae;g__Sclerotinia;s__Sclerotinia_sclerotiorum
49076be858535133180b0c688159899a	787	TRUE	55	90	128	198	604	1	1	1	4	15	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;
4eac723a6a9e8217f28c2ab549602833	785	TRUE	1	1	1	1	21	242	403.5	603	992.5	1965	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
f6319c80505cfc39f49e0fdbb1f64b7e	783	TRUE	1	1	1	1	1	47	132	222	719.5	9371	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora
5e557a68e85ee03eaebc52062dcdff98	782	TRUE	1	6	10	16.5	28	721	1856.5	2341	4152	5703	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Xylariales
568dbc1d0aad7dde78fe451cee3261c3	782	TRUE	1	1	1	1	1	47	128.5	165	217.5	429	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
3836f16615f65eff08ee4dbf92177a5b	780	TRUE	543	1098	2169	3583.5	13611	39	49	95	112	171	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae
fd117529a30825ea0f76b6ca7ea0aa1e	776	TRUE	1	1	1	1	1	45	66	94	310	1023	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__unidentified
dd9a6a4f9596f0541b4001eb9d6f4fbf	776	TRUE	1	1	1	16.5	25	235	427	601	742.5	2027	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
8a5726aa2c7721a33c6cf40a51a920b5	775	TRUE	1	1	1	1	11	46	82	96	153	545	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
cbbb5beb9ee83e5bcf8016569a53e433	774	TRUE	1	1	1	1	13	34	65	70	107.5	168	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae
5de7fb9292106c74a78cb262b9cfd7c3	773	TRUE	1	1	1	1	20	31	52.5	77	160.5	198	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporales_fam_Incertae_sedis;g__Chalastospora;s__Chalastospora_gossypii
83cbd2de93b9aa5206ed87138fc957a1	773	TRUE	35	54	93	127	364	1	5.5	10	14.5	26	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Hannaella;s__Hannaella_coprosmae
5e601f1cbc8b00d3a58e7d6d1f39baae	772	TRUE	16	132.5	220	336.5	833	1	2.5	11	38.5	153	k__Fungi;p__Ascomycota;c__Sordariomycetes
82516ed0f0cfbddd67f45d1693baa15f	770	TRUE	1	8.5	13	28	103	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Bionectriaceae;g__Clonostachys;s__Clonostachys_miodochialis
957bd35c269036eb911a617463617201	770	TRUE	1	61.5	105	142.5	311	1	1	1	1	46	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
d7952e7c56063e10003919d4dce7a53b	769	TRUE	1	1	1	1	81	56	132	226	506.5	906	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporales_fam_Incertae_sedis;g__Chalastospora;s__Chalastospora_gossypii
fad4e28e32b38df9f6b7216dee063980	767	TRUE	1	1	1	1	234	58	78.5	118	303	648	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Bipolaris
84843c411ce2b93404f860cd2bcf44d7	764	TRUE	1	1	1	1	6	10	16.5	44	62.5	127	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Mycosphaerellaceae;g__Zymoseptoria;s__Zymoseptoria_brevis
7f40b66bb807c21df060122a140e98c1	763	TRUE	1	1	1	1	8	1	45	57	114.5	156	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Pyrenophora;s__Pyrenophora_tritici-repentis
18759eb25f201572792b395a9e8cffee	761	TRUE	182	411	560	834.5	1914	1	48	79	107.5	373	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Stemphylium;s__Stemphylium_vesicarium
6c139348b0f7299ea8f38436467ccab0	760	TRUE	1	1	1	2.5	18	21	48	93	149.5	196	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporales_fam_Incertae_sedis;g__Chalastospora;s__Chalastospora_gossypii
e523e1fc4b8a76c0983a1bfb55fbb3c5	759	TRUE	1	1	3	6	21	36	39.5	70	93.5	121	k__Fungi
2119b24c0bba7c01ab5ed649948d64d0	751	TRUE	72	153	185	283.5	408	1061	1774	3207	5975	6765	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporales_fam_Incertae_sedis;g__Chalastospora;s__Chalastospora_gossypii
d0f63ae4057c8d978e554cc723b10414	748	TRUE	133	161	249	272.5	514	983	1930	2951	4479.5	5248	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
bbd410c02034a7911f096abf70d5dfad	748	TRUE	1	72.5	175	279.5	8290	3455	4455.5	7661	9333	10944	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
bfb189f6ade06c5933ccb7c6aa29495d	738	TRUE	43	63	89	147.5	518	307	576.5	910	1614.5	1835	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Sporobolomyces;s__Sporobolomyces_ruberrimus
f64349490a3d8a5573ec60e996649023	738	TRUE	50	76	114	174.5	207	461	736.5	828	1081.5	1638	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__unidentified
d930c46706f8f0136922bcba2c0e691f	736	TRUE	1	1	1	1	1	1	14.5	20	38.5	101	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales
61726155e3168b7f07b15d4459b8c716	735	TRUE	1	1	1	1	30	1	15	29	46	440	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
7bd3c3df5b7a80c46e14ad8f5e6be892	729	TRUE	1	1	1	1	9	1	35	53	87	167	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Rhodotorula
6faf238e18bcc6a7d6810fc0509c3c3f	718	TRUE	1	1	1	1	4	1	11.5	19	65.5	96	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Dothideales;f__Dothideales_fam_Incertae_sedis;g__Selenophoma;s__Selenophoma_mahoniae

Table D.4 cont. Differentially abundant fungal ASVs identified by ANCOM when comparing the seed microbiomes of three agricultural crops grown in Saskatoon, Canada in 2016.

ASV	**W	*Reject null			CanolaOff					LentilOff			
Percentiles			0	25	50	75	100	0	25	50	75	100	Taxonomy
ebc9a61cf82bc67fe1c129fcbd96592f	816	TRUE	1	1	1	1	1	281	2346	3472	6998.5	21798	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Helotiales;f__Sclerotiniaceae
0d8f1c3f3e4425b3d40726c1edf7f937	816	TRUE	1	1	1	1	1	1638	3016	3759	5321.5	8493	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Glomerellales;f__Glomerellaceae;g__Colletotrichum;s__Colletotrichum_lentis
325b04fce747ff9791507c60555994eb	816	TRUE	526	1768.5	3828	8795	21909	1	1	1	1	5	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Leptosphaeriaceae;g__unidentified;s__unidentified
81dd4b112b3a91da16e30434fcd7ba1e	815	TRUE	2310	3654	5338	8120.5	11940	1	1	1	8.5	54	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_brassicae
5d3e3dfd137f3b94cea23f71de637b0d	814	TRUE	388	1201	1596	1758	4013	1	1	1	1	19	k__Fungi;p__Basidiomycota;c__Exobasidiomycetes;o__Entylomatales;f__unidentified;g__unidentified;s__unidentified
1ac7001628cd5926185b585586fb058d	813	TRUE	1	1	1	1	8	81	124	203	296	442	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Rhynchogastremataceae;g__Papiliotrema
0673bdbc8a6e165af5af2c8689302d70	812	TRUE	168	543	725	765.5	1388	1	1	1	11	47	k__Fungi;p__Basidiomycota;c__Exobasidiomycetes;o__Entylomatales;f__unidentified;g__unidentified;s__unidentified
d9f58908e54fea3618f22844d0c556a6	812	TRUE	21	107	128	152	2598	1	1	1	1	11	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_oudemansii
49076be858535133180b0c688159899a	811	TRUE	1	1	1	3.5	19	55	90	128	198	604	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
d7952e7c56063e10003919d4dce7a53b	809	TRUE	76	148.5	191	259	309	1	1	1	1	81	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporales_fam_Incertae_sedis;g__Chalastospora;s__Chalastospora_gossypii
f034ec7258574248184222 28b7fe8b54	807	TRUE	27	85	131	171	1373	1578	3203	4649	8353.5	11914	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
75a9b73903a3053226dff54454c05cd9	807	TRUE	1	50	133	273	871	1	1	1	1	3	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Erysiphales;f__Erysiphaceae;g__Blumeria;s__Blumeria_graminis
c39ddb3469eef1b997ee59c419b57d26	806	TRUE	1	48	92	218	658	736	2151	3452	10282.5	21719	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Helotiales;f__Sclerotiniaceae;g__Sclerotinia;s__Sclerotinia_sclerotiorum
5de7fb9292106c74a78cb262b9cfd7c3	806	TRUE	31	37.5	52	67	76	1	1	1	1	20	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporales_fam_Incertae_sedis;g__Chalastospora;s__Chalastospora_gossypii
9d6eddf36e03d4d20c1ad1cdfa130688	800	TRUE	1	32	80	431.5	7068	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Trichothecium;s__Trichothecium_roseum
3836f16615f65eff08ee4dbf92177a5b	799	TRUE	53	75	134	232	278	543	1098	2169	3583.5	13611	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae
9e8d9b660109920ab6f616992e440457	798	TRUE	1	1	1	1	1	1	13	180	230.5	861	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
a9be25c1f5877c27b288acf18dd6e97f	797	TRUE	1	20.5	121	2485.5	16683	1	1	1	1	6	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Eurotiales;f__Aspergillaceae;g__Aspergillus;s__Aspergillus_proliferans
2119b24c0bba7c01ab5ed649948d64d0	794	TRUE	1384	2320.5	2973	3352.5	6017	72	153	185	283.5	408	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporales_fam_Incertae_sedis;g__Chalastospora;s__Chalastospora_gossypii
8f26708a09ff3158fcfc07e801eddf70	793	TRUE	677	1889	2637	3234	6012	41	92	128	189.5	553	k__Fungi;p__Basidiomycota;c__Exobasidiomycetes;o__Entylomatales;f__Entylomatales_fam_Incertae_sedis;g__Tilletiopsis;s__Tilletiopsis_washingtonensis
82516ed0f0cfbddd67f45d1693baa15f	792	TRUE	1	1	1	1	1	1	8.5	13	28	103	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Bionectriaceae;g__Clonostachys;s__Clonostachys_miodochialis
6c139348b0f7299ea8f38436467ccab0	789	TRUE	11	28	35	51	71	1	1	1	2.5	18	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporales_fam_Incertae_sedis;g__Chalastospora;s__Chalastospora_gossypii
61726155e3168b7f07b15d4459b8c716	787	TRUE	1	19.5	36	47.5	94	1	1	1	1	30	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Trichosphaeriales;f__Trichosphaeriaceae;g__Nigrospora;s__Nigrospora_oryzae
d930c46706f8f0136922bcba2c0e691f	762	TRUE	1	13.5	22	38	61	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales
5dfdda12a5800fb11bc3ee736f2df12a	744	TRUE	1	4	22	40	95	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Leptosphaeriaceae;g__Ampelomyces;s__Ampelomyces_quisqualis

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

APPENDIX E: Taxonomy obtained from endophytic samples sequencing.

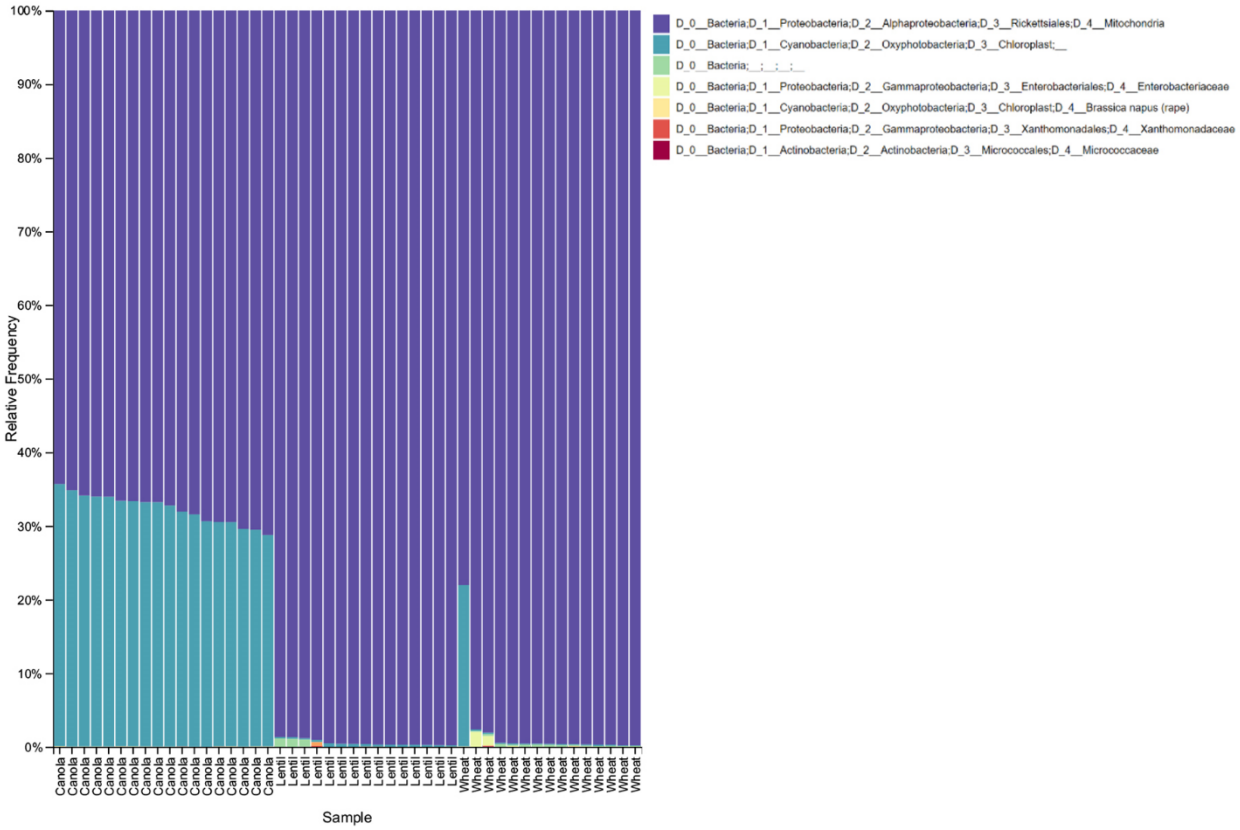

Figure E.1 Taxonomy obtained from endophytic samples sequencing.

APPENDIX F: Precipitation (mm) during growing season in 4 site years analyzed in Saskatchewan, Canada.

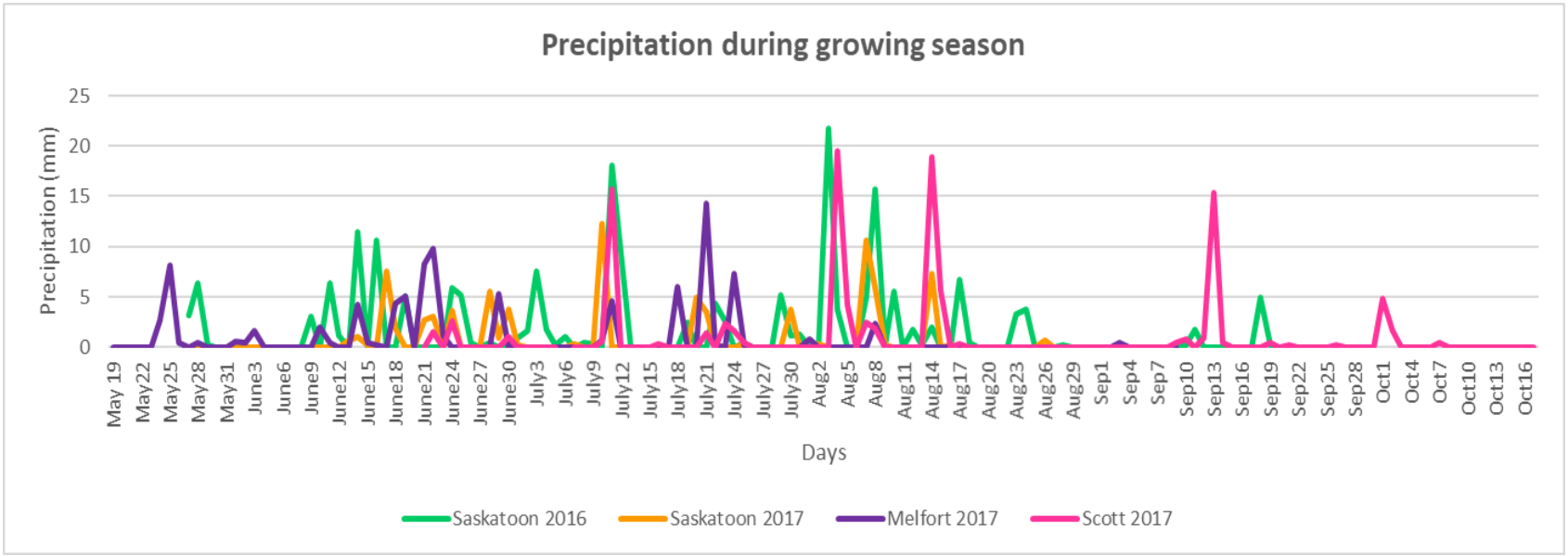

Figure F.1 Precipitation (mm) during growing season in four site years analyzed in Saskatchewan, Canada. Scott plots were initially seeded in May, but they were lost due to a hailstorm, consequently, plots were reseeded later in June (Taye et al. 2020; Bazghaleh et al. 2020).

APPENDIX G: Venn diagrams depicting ASVs detected in the canola seed microbiome across two years and three locations in Saskatchewan.

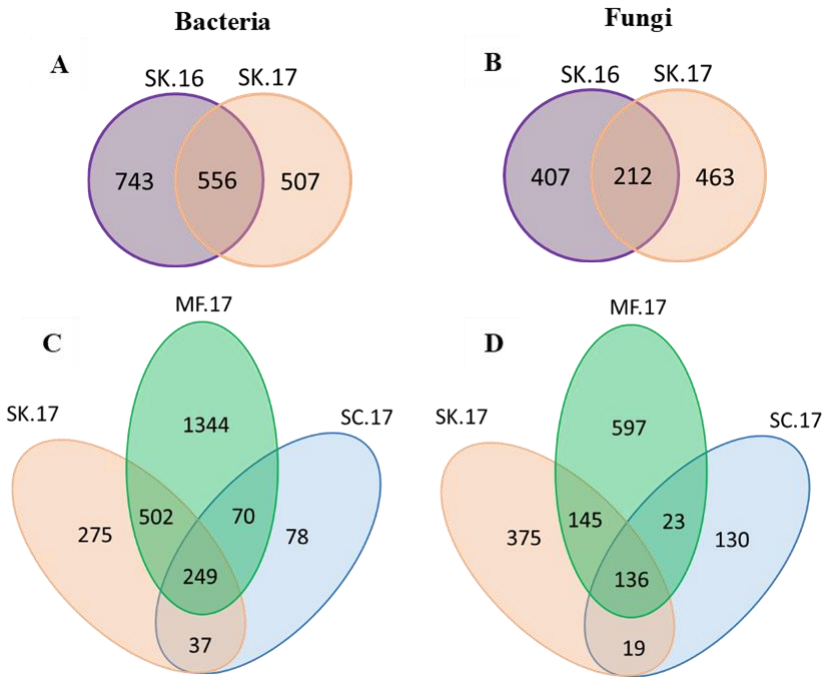

Figure G.1 Venn diagrams depicting ASVs detected in the *Brassica napus* seed microbiome across two years **(A, B)** and three locations **(C, D)** in Saskatchewan. ASVs found in not all but at least one of the replicates of each sample examined.

APPENDIX H: α-Diversity estimators of *Brassica napus* seed bacterial and fungal microbiomes.

Table H.1 α-Diversity estimators of *Brassica napus* seed bacterial and fungal microbiomes from two years in Saskatoon.

	Bacteria				**Fungi**			
	Chao 1*		**Inverse Simpson's***		**Chao 1***		**Inverse Simpson's***	
Line	SK 2016 **A**	SK 2017 **B**	SK 2016 **A**	SK 2017 **B**	SK 2016	SK 2017	SK 2016 **A**	SK 2017 **B**
NAM-0	269	71.4	12.0 **ab**	4.5	109.3	152.7 **a**	10.4 **a**	14.0 **a**
NAM-13	216.7	178.9	11.6 **ab**	9.9	107.3	105.7 **ab**	7.2 **ab**	5.5 **b**
NAM-17	181.2	271.8	8.5 **ab**	7.4	102.7	106.8 **ab**	7.4 **ab**	4.8 **bc**
NAM-32	113.8	55.5	4.1 **b**	5.8	101.7	74.7 **b**	9.8 **ab**	3.8 **cd**
NAM-37	321.2	51.2	6.8 **ab**	2.5	101.7	117.3 **ab**	11.1 **a**	5.6 **b**
NAM-43	153.6	67.6	6.4 **ab**	2.5	84	88 **b**	4.6 **b**	3.6 **cd**
NAM-72	164.8	60	3.2 **b**	4.5	88.3	104.7 **ab**	7.6 **ab**	4.1 **cd**
NAM-94	152.2	153.7	14.8 **a**	3.8	94	79.3 **b**	7.7 **ab**	2.9 **d**

*Tukey post-hoc test ($p < 0.05$). Lowercase letters represent differences among lines within a field. Uppercase letters represent differences between years.

APPENDIX I: α-Diversity estimators of *Brassica napus* seed bacterial and fungal microbiomes from three locations in Saskatchewan.

Table I.1 α-Diversity estimators of *Brassica napus* seed bacterial and fungal microbiomes from three locations in Saskatchewan.

	Bacteria						Fungi					
	Chao 1*			Inverse Simpson's*			Chao 1*			Inverse Simpson's*		
Line	SK 2017 **B**	MF 2017 **A**	SC 2017 **B**	SK 2017 **B**	MF 2017 **A**	SC 2017 **B**	SK 2017 **B**	MF 2017 **A**	SC 2017 **C**	SK 2017 **C**	MF 2017 **A**	SC 2017 **B**
NAM-0	71.4	724.1	57.4	4.5	55.3	5.8	152.7 **a**	80.7 **b**	104 **a**	14.0 **a**	8.3 **c**	13.4
NAM-13	178.9	409.3	103.5	9.9	27.9	6.3	105.7 **ab**	157 **a**	81 **ab**	5.5 **b**	13.3 **a**	10.4
NAM-17	271.8	460.4	90.7	7.4	19.9	7.8	106.8 **ab**	132.7 **ab**	85.7 **ab**	4.8 **bc**	13.1 **a**	8.3
NAM-32	55.5	452.2	59.2	5.8	8.8	3.2	74.7 **b**	138 **a**	98.3 **ab**	3.8 **cd**	12.6 **ab**	10.3
NAM-37	51.2	474.5	76.6	2.5	9.4	7.1	117.3 **ab**	146.3 **a**	62.7 **b**	5.6 **b**	12.7 **ab**	6.9
NAM-43	67.6	454.3	71.9	2.5	14.6	4.3	88 **b**	134.3 **a**	79.7 **ab**	3.6 **cd**	10.5 **bc**	6.9
NAM-72	60	606.3	72.4	4.5	25.5	4.5	104.7 **ab**	163.3 **a**	75.3 **ab**	4.1 **cd**	12.9 **a**	9.5
NAM-94	153.7	626.7	72.9	3.8	30.3	5.2	79.3 **b**	140 **a**	84.3 **ab**	2.9 **d**	12.6 **ab**	8.7

*Tukey post-hoc test ($p < 0.05$). Lowercase letters represent differences among lines within a field. Uppercase letters represent differences among locations.

APPENDIX J: Differentially abundant bacterial and fungal ASVs identified by ANCOM when comparing *Brassica napus* seed microbiomes across two years in Saskatoon and three locations in 2017.

Table J.1 Differentially abundant bacterial ASVs identified by ANCOM when comparing *Brassica napus* seed microbiomes across two years in Saskatoon.

ASV	**W	*Reject null	SK16					SK17					
			0	25	50	75	100	0	25	50	75	100	Taxonomy
674330d2712cf4dece0dee9c4fa4902b	1801	TRUE	1	103	833	2753.75	11513	1	1	1	1	112	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Erwinia
531efdc4c2102566dbec467775e4da8f	1797	TRUE	5	45	155	247	2242	1	1	1	3	47	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Rhizobiaceae
99b3f0f472a923d6a905a01f0d836e2b	1781	TRUE	1	1	1	6	29	1	6	15.5	102.25	968	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Buchnera
5b232e03bbd44cf5bfd9b460cc763dd9	1780	TRUE	1	1	1	1	12	1	3	8	20	270	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Serratia
d3af4a709780e098a4292f4397f55ede	1779	TRUE	35	81.5	146	259.5	1308	1	1	6.5	14	153	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium
d8dd0705dd011456cefd4e3ac9a840e6	1777	TRUE	4	21.5	36	66.25	358	1	1	1	2.25	30	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;D_5__Falsirhodobacter
10f50071098e6cca71f48e435706a1d5	1771	TRUE	8	69.5	156	322.75	2744	1	1	2.5	8.75	781	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria
591650397089e03dfe873be0226b5532	1768	TRUE	3	39	63	95.75	677	1	1	1	6	70	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Saccharibacillus
2d8695f45cac585649776080c6e2a55b	1767	TRUE	5	45.25	82.5	276.25	979	1	1	1	5	308	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Paenibacillus
d949992d2ca07058a81a858288683193	1764	TRUE	1	15	28	88.25	1415	1	1	1	1	5	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Xanthomonadales;D_4__Xanthomonadaceae;D_5__Stenotrophomonas
d78003f13712da48d0bb36fd45116b91	1757	TRUE	1	6.75	14.5	28.5	245	1	1	1	1	4	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Caulobacterales;D_4__Caulobacteraceae
f88bf228d27bdeb62a0863534da0745e	1756	TRUE	1	10.25	20	54.75	339	1	1	1	1	21	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Weeksellaceae;D_5__Chryseobacterium
555150f7f4b6ef9a9db9861501dd51cf	1754	TRUE	4	16	34	63	190	1	1	1	4.25	47	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
327d4d6ce0e897b5da757c92ef0f4e00	1753	TRUE	1	16.5	51.5	65.25	359	1	1	1.5	3	42	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae
429f14995ac82e261139a3f9cc06e6b3	1744	TRUE	11	28	49	209	587	1	1	4	8.25	97	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Rhizobiaceae;D_5__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
a6580129a3ace1a83e446dca31617824	1737	TRUE	81	234	295.5	429	1758	5	13.5	30.5	78.75	505	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
a78502abc40f13bb7549bd4be6ab45c2	1736	TRUE	1	5.5	12.5	34.75	190	1	1	1	1	7	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
1bad9c540ef9ad2949aa0623110d86c4	1731	TRUE	2	8	14	35.5	125	1	1	1	3	19	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
73fed31595dbfab1585b1f7affb05498	1728	TRUE	4	8.75	14	26	73	1	1	1	4.25	13	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales
cce70d5962057843ac2fbadc53741e55	1728	TRUE	111	239.25	442.5	885	2630	6	23.25	55	107.5	741	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae
94dbd0eda2f2563cdf95d7cd68e2ec4b	1725	TRUE	5	15.75	25.5	48	203	1	1	2.5	6	62	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Rathayibacter
192c5a817f48368339e519e0a0a907da	1725	TRUE	1	1	1	1	6	1	1	3.5	6.25	492	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Serratia
0f7064630dae097a6ad884f59e1fd4c3	1723	TRUE	1	7	11.5	26.25	95	1	1	1	1	9	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
f7bb8f7f10b371377fe7fde66196b940	1721	TRUE	1	3.75	11	33	449	1	1	1	1	5	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae
b9dd335eea215a09208207fd65fbd79c	1713	TRUE	1	4.75	10	20.25	90	1	1	1	1	14	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__Pedobacter
98bcb1b74afd32517170c3da64daf523	1711	TRUE	1	17.5	71.5	279.25	1176	1	1	1	1.5	598	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Paenibacillus
3c66e2899f73859d4cd81e0407cfbe49	1711	TRUE	1	4	7.5	12.25	131	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Myxococcales
50e5357d1ca7696eb3487cb71787b9d5	1700	TRUE	1	9	12	23.5	136	1	1	1	1.5	26	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Variovorax
64eb2494dbf4892b1fcfc8aae01b7158	1689	TRUE	1	8.5	20.5	38.5	376	1	1	2.5	5	28	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingomonas
fbb68742aac7d6e4dc1589359e99f1f5	1688	TRUE	1	3	7	14.25	48	1	1	1	1	8	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Weeksellaceae;D_5__Chryseobacterium
545f313453aaeef5c994971a2c8f7148	1672	TRUE	1	2.75	5	13.25	32	1	1	1	1	4	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__Pedobacter
d0ab5f3fd572303d47328ebf7db7ba18	1667	TRUE	1	3	9.5	15	152	1	1	1	1	10	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Variovorax
f14f8d4035339bd7ced048bc8b8dbeab	1663	TRUE	1	3.75	5	15.75	46	1	1	1	1	4	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Weeksellaceae;D_5__Chryseobacterium
28e2c2e581b768b883d582c50e87e885	1655	TRUE	1	3.75	14	39.5	445	1	1	1	1	34	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Xanthomonadales;D_4__Xanthomonadaceae;D_5__Stenotrophomonas
a3f5dafdc70e6e2896b40966022afbea	1643	TRUE	1	5.25	15	31.25	230	1	1	1	1.5	7	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Xanthomonadales;D_4__Xanthomonadaceae;D_5__Stenotrophomonas;D_6__Stenotrophomonas chelatiphaga
3f91f9bdfb7c2ebeb41e74fcf1d22eaa	1628	TRUE	1	3	4	12	37	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Verticia

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

Table J.2 Differentially abundant fungal ASVs identified by ANCOM when comparing *Brassica napus* seed microbiomes across two years in Saskatoon.

ASV	**W	*Reject null	SK16					SK17					
			0	25	50	75	100	0	25	50	75	100	Taxonomy
0673bdbc8a6e165af5af2c8689302d70	1081	TRUE	101	497.25	581	756.25	1380	1	1	1	1	11	k__Fungi;p__Basidiomycota;c__Exobasidiomycetes;o__Entylomatales
5d3e3dfd137f3b94cea23f71de637b0d	1081	TRUE	166	1245.75	1505	1681.75	3988	1	1	1	1	10	k__Fungi;p__Basidiomycota;c__Exobasidiomycetes;o__Entylomatales
63d3734f6c084466d644f3dd20ebca8b	1077	TRUE	1	1	1	1	1	1	20.5	41.5	70	309	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae
c39ddb3469eef1b997ee59c419b57d26	1073	TRUE	1	30	78.5	317	1010	1	1	1	2	52	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Helotiales;f__Sclerotiniaceae
8f26708a09ff3158fcfc07e801eddf70	1072	TRUE	290	1306.75	2121.5	3050	5916	9	36	62	127.25	412	k__Fungi;p__Basidiomycota;c__Exobasidiomycetes;o__Entylomatales;f__Entylomatales_fam_Incertae_sedis;g__Tilletiopsis;s__Tilletiopsis_washingtonensis
fad4e28e32b38df9f6b7216dee063980	1072	TRUE	1	1	1	1	141	1	35.75	71	130.75	482	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Bipolaris;s__Bipolaris_maydis
9d6eddf36e03d4d20c1ad1cdfa130688	1072	TRUE	1	38	69.5	211.5	7041	1	1	1	1.75	20	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Trichothecium;s__Trichothecium_roseum
325b04fce747ff9791507c60555994eb	1068	TRUE	522	1855.75	3940	8427.75	33468	4	62.5	150	253.75	9135	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Leptosphaeriaceae
ba095f1690a3b11630aace84c78cb556	1068	TRUE	1	1	1	1	113	1	49.75	87.5	106.5	221	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Mycosphaerellaceae;g__Mycosphaerella;s__Mycosphaerella_tassiana
75a9b73903a3053226dff54454c05cd9	1066	TRUE	1	28	57	196.5	868	1	1	1	1	168	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Erysiphales;f__Erysiphaceae;g__Blumeria;s__Blumeria_graminis
386e54fe292c2f421b3f407434f6d9b8	1061	TRUE	1	1	232.5	430.5	1467	1	1	1	1	131	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Glomerellales;f__Plectosphaerellaceae;g__Plectosphaerella;s__Plectosphaerella_oratosquillae
f3f9d51940df8c58e5442d785cd279b7	1060	TRUE	1	1	59	108.25	420	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_brassicae
7eb776a43a89eb41525e639fdeb54b43	1060	TRUE	1	6.25	27	48.5	216	1	1	1	1	3	k__Fungi;p__Ascomycota;c__Sordariomycetes
1d859da4552f8aac14ac4ce577a1ce7f	1059	TRUE	1	1	1	1	12	1	11.5	26.5	53	1412	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Eurotiales;f__Aspergillaceae;g__Aspergillus
ea295802e3087f6861ac6b934a9a9543	1058	TRUE	1	5.75	13.5	23	47	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Dissoconiaceae;g__Dissoconium;s__Dissoconium_aciculare
5dfdda12a5800fb11bc3ee736f2df12a	1050	TRUE	1	1	11	36.25	91	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Leptosphaeriaceae;g__Ampelomyces;s__Ampelomyces_quisqualis
bbd410c02034a7911f096abf70d5dfad	1046	TRUE	17	88.25	119	249	5080	1	10.25	20.5	57	601	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae
b868159fd517bf07e6182e1c608948a8	1026	TRUE	1	1	1	7.25	45	1	16.75	22	47.75	92	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Dothideales;f__Aureobasidiaceae;g__Aureobasidium;s__Aureobasidium_pullulans
81dd4b112b3a91da16e30434fcd7ba1e	1006	TRUE	900	3647.75	5701.5	9228.25	13552	30	1000.5	1803.5	2213.75	3224	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_brassicae
a849475488750a200272695826d74b12	974	TRUE	1	2.5	5.5	11.75	315	1	1	1	1	13	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Glomerellales;f__Plectosphaerellaceae;g__Lectera;s__Lectera_longa
7313efe22060402792d7042e44d3282c	974	TRUE	1	1	1	1	8	1	1	4	8.5	112	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Mrakiaceae;g__Itersonilia;s__Itersonilia_pannonica

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

Table J.3 Differentially abundant bacterial ASVs identified by ANCOM when comparing *Brassica napus* seed microbiomes from samples harvested in Saskatoon and Melfort during 2017.

ASV	**W	*Reject null	MF17					SK17					
			0	25	50	75	100	0	25	50	75	100	Taxonomy
a6cb5951f81c2d6f6f7c303b4df53140	2472	TRUE	11	26.75	359.5	660.75	1145	1	1	3	5.5	106	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Propionibacteriales;D_4__Propionibacteriaceae;D_5__Microlunatus
6f4e6f3e07346f7b98305b599389833f	2469	TRUE	1	1	1	1.5	43	1	10.25	56	165.25	13810	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Rosenbergiella
99b3f0f472a923d6a905a01f0d836e2b	2469	TRUE	1	1	1	1	1	1	6	15.5	102.25	968	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Buchnera
db2e2e1484d09bcb17556b04ec894c65	2462	TRUE	1	1	1	1	62	1	8.75	27	172.25	13198	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
893d12123eb7f623489a4f025a15ae7f	2442	TRUE	1	131.25	283.5	575	6085	162	1193.5	1996	2468.5	6888	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae
0f5c88b2e79c36e9a80b20bc80c998b1	2427	TRUE	8	32	130.5	270.25	670	1	1	3	7	206	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Bacillaceae;D_5__Bacillus
2522c23ffe5424b561a0a42d3d229a4e	2413	TRUE	1	6.5	40	85.75	161	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Rubrobacteria;D_3__Rubrobacterales;D_4__Rubrobacteriaceae;D_5__Rubrobacter
3d3cc88489d58b914d4eb0a92d5b9de9	2412	TRUE	1	8.75	47.5	117.5	210	1	1	1	1	10	D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Solibacterales;D_4__Solibacteraceae;D_5__Bryobacter
143dc20909a29e97d1b8ab9c04876a8c	2393	TRUE	1	4	37.5	71.5	160	1	1	1	1	3	D_0__Bacteria;D_1__Actinobacteria;D_2__Rubrobacteria;D_3__Rubrobacterales;D_4__Rubrobacteriaceae;D_5__Rubrobacter
c9de781a5fcb7e51394be942835afc3f	2370	TRUE	28	205.75	588	945.5	1759	1	8.5	17	42	209	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Corynebacteriales;D_4__Nocardiaceae;D_5__Rhodococcus
5d675a3518222fc99c8cba34feaeac81	2347	TRUE	1	3.5	29.5	79.5	156	1	1	1	1	4	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia;D_3__Gaiellales
d292539ba659aac837e2770682eda4a2	2337	TRUE	1	3.75	22.5	55.75	101	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia;D_3__Gaiellales
d48d6db680d3f7b5bee737aff5727286	2332	TRUE	1	4	34.5	68.5	231	1	1	1	1	12	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Frankiales;D_4__Geodermatophilaceae
4ea2bee24a8209f99a0c8e60537d4e5c	2327	TRUE	1	5	17.5	56.75	99	1	1	1	1	16	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Acetobacterales;D_4__Acetobacteraceae
bc02cccf2504e897a3b969f86990f28b	2297	TRUE	1	4	37	94.25	153	1	1	1	3	38	D_0__Bacteria;D_1__Actinobacteria;D_2__Rubrobacteria;D_3__Rubrobacterales;D_4__Rubrobacteriaceae;D_5__Rubrobacter
4456598990b6bc58aeee15f041f258a7	2295	TRUE	1	6.5	21.5	73.75	216	1	1	1	1.25	12	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Bacillaceae;D_5__Bacillus
b2af163540d17007833dc819704afb28	2291	TRUE	1	1.75	51	82.25	212	1	1	1	1	14	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Frankiales
d3af4a709780e098a4292f4397f55ede	2287	TRUE	18	72.75	91.5	144.25	284	1	1	6.5	14	153	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium
113bb511f2e84ff8c0842b9a5ca6f31a	2285	TRUE	1	4	15	24.75	98	1	1	1	1	1	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Chitinophagales;D_4__Chitinophagaceae;D_5__Segetibacter
6de3541f0af38b80fbfe4fdf7e7b4dc4	2277	TRUE	2	8.25	15	23.25	718	1	1	1	3	5	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Micrococcaceae
9311bffe5a78c0df3d0077d5874d6b47	2276	TRUE	1	4	15.5	32.75	63	1	1	1	1	7	D_0__Bacteria;D_1__Chloroflexi;D_2__Chloroflexia;D_3__Thermomicrobiales
5e582cb9d5f776caa8ed92f775680b66	2255	TRUE	1	3	11.5	26.75	83	1	1	1	1	3	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Microvirga
1ce88f3f29ece47ec66e85b1b9ec5f1b	2252	TRUE	1	1	17	50.75	95	1	1	1	1	9	D_0__Bacteria;D_1__Chloroflexi
1f5025eeff0b2734a9ae6fdcb994fdc5	2246	TRUE	1	8.5	15.5	22.75	73	1	1	1	1.25	10	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium
1234621434ce4571bb24bc90dab0530f	2244	TRUE	1	4	12.5	24	63	1	1	1	1	5	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Streptosporangiales;D_4__Streptosporangiaceae
ec73d0a13e9c39fecb034ef61681d3d0	2240	TRUE	1	3.75	22	65.5	99	1	1	1	1.25	23	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Propionibacteriales;D_4__Nocardioidaceae
80ab331c858f44e438a319a84298facc	2238	TRUE	1	4.75	13	24.75	79	1	1	1	1	8	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae
dd9c00c47bd965236dc8c5bddd25f15e	2237	TRUE	1	3.25	11	44	92	1	1	1	1	3	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Propionibacteriales;D_4__Nocardioidaceae
79c7b38c4902075c1b5f57d507a630e8	2228	TRUE	1	3.5	26	46.75	84	1	1	1	1	9	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia;D_3__Solirubrobacterales;D_4__Solirubrobacteraceae;D_5__Solirubrobacter

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

Table J.4 Differentially abundant bacterial ASVs identified by ANCOM when comparing *Brassica napus* seed microbiomes from samples harvested in Saskatoon and Scott during 2017.

ASV	**W	*Reject null	SC17					SK17					
			0	25	50	75	100	0	25	50	75	100	Taxonomy
674330d2712cf4dece0dee9c4fa4902b	1210	TRUE	5	79	638.5	1435.25	7984	1	1	1	1	112	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Erwinia
db2e2e1484d09bcb17556b04ec894c65	1210	TRUE	267	698.25	2276	3963.75	5697	1	8.75	27	172.25	13198	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
285172e1225e4e0c22f05d6744764d9c	1205	TRUE	7	10.75	13.5	18.25	59	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Ralstonia
6f4e6f3e07346f7b98305b599389833f	1201	TRUE	1	1	1	1	13	1	10.25	56	165.25	13810	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Rosenbergiella
7a89fbcc332cdb3e7459401950594ae5	1194	TRUE	3	5	7	10	16	1	1	1	1	3	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Acetobacterales;D_4__Acetobacteraceae
893d12123eb7f623489a4f025a15ae7f	1187	TRUE	1	47.25	176.5	555	2058	162	1193.5	1996	2468.5	6888	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae
5b232e03bbd44cf5bfd9b460cc763dd9	1187	TRUE	1	1	1	1	1	1	3	8	20	270	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Serratia
8bf175f329d16e4aa732cf2b32279df3	1181	TRUE	25	178.25	399	1103	3329	235	1343.5	4344.5	6446.75	16095	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae
2d8695f45cac585649776080c6e2a55b	1168	TRUE	3	10	27.5	56.75	586	1	1	1	5	308	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Paenibacillaceae;D_5__Paenibacillus
7f4dd5b95ce86f83c2dc25b33f0e87b8	1162	TRUE	1	1	1	1	7	1	1.75	4	33.5	169	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Enterococcaceae;D_5__Enterococcus;D_6__Enterococcus mundtii
5fd2507be8d352399835cc82a000e46e	1161	TRUE	1	2.5	7	14	47	1	1	1	1	6	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
a6580129a3ace1a83e446dca31617824	1157	TRUE	35	75	135	328.5	1334	5	13.5	30.5	78.75	505	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae
f85e0ad6ec1269099703d64c81c3366e	1155	TRUE	1	1	1	1	5	1	1.75	4.5	20.5	272	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Buchnera
192c5a817f48368339e519e0a0a907da	1138	TRUE	1	1	1	1	1	1	1	3.5	6.25	492	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Serratia

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

Table J.5 Differentially abundant bacterial ASVs identified by ANCOM when comparing *Brassica napus* seed microbiomes from samples harvested in Melfort and Scott during 2017.

ASV	**W	*Reject null	MF17					SC17					
			0	25	50	75	100	0	25	50	75	100	Taxonomy
db2e2e1484d09bcb17556b04ec894c65	2279	TRUE	1	1	1	1	62	267	698.25	2276	3963.75	5697	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
674330d2712cf4dece0dee9c4fa4902b	2279	TRUE	1	1	1	1	212	5	79	638.5	1435.25	7984	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Erwinia
285172e1225e4e0c22f05d6744764d9c	2278	TRUE	1	1	1	1	1	7	10.75	13.5	18.25	59	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Ralstonia
a6cb5951f81c2d6f6f7c303b4df53140	2278	TRUE	11	26.75	359.5	660.75	1145	1	1	1	1	4	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Propionibacteriales;D_4__Propionibacteriaceae;D_5__Microlunatus
d980f5679cc5d3a0aa27a34284b9bc3b	2259	TRUE	1	1	1	1	11	1	4	8	21.5	58	D_0__Bacteria;D_1__Tenericutes;D_2__Mollicutes
fc1d9940419420113ce3fbfacc8d703a	2245	TRUE	80	132.25	229	398.75	6817	1	2	6	19.75	64	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Micrococcaceae
3d3cc88489d58b914d4eb0a92d5b9de9	2233	TRUE	1	8.75	47.5	117.5	210	1	1	1	1	3	D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Solibacterales;D_4__Solibacteraceae (Subgroup 3);D_5__Bryobacter
99b3f0f472a923d6a905a01f0d836e2b	2228	TRUE	1	1	1	1	1	1	1	4.5	10.5	30	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Buchnera
a05e528b1e056246195f3581b8b5f591	2217	TRUE	1	1	1	1	1	1	1	4	6.25	247	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Pseudonocardiales;D_4__Pseudonocardiaceae;D_5__Saccharopolyspora
2522c23ffe5424b561a0a42d3d229a4e	2209	TRUE	1	6.5	40	85.75	161	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Rubrobacteria;D_3__Rubrobacterales;D_4__Rubrobacteriaceae;D_5__Rubrobacter
c9de781a5fcb7e51394be942835afc3f	2206	TRUE	28	205.75	588	945.5	1759	1	4.75	11	27.5	135	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Corynebacteriales;D_4__Nocardiaceae;D_5__Rhodococcus
bc02cccf2504e897a3b969f86990f28b	2198	TRUE	1	4	37	94.25	153	1	1	1	1	4	D_0__Bacteria;D_1__Actinobacteria;D_2__Rubrobacteria;D_3__Rubrobacterales;D_4__Rubrobacteriaceae;D_5__Rubrobacter
0f5c88b2e79c36e9a80b20bc80c998b1	2184	TRUE	8	32	130.5	270.25	670	1	1	4.5	12.25	38	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Bacillaceae;D_5__Bacillus
143dc20909a29e97d1b8ab9c04876a8c	2174	TRUE	1	4	37.5	71.5	160	1	1	1	1	4	D_0__Bacteria;D_1__Actinobacteria;D_2__Rubrobacteria;D_3__Rubrobacterales;D_4__Rubrobacteriaceae;D_5__Rubrobacter
5d675a3518222fc99c8cba34feaeac81	2165	TRUE	1	3.5	29.5	79.5	156	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia;D_3__Gaiellales
b2af163540d17007833dc819704afb28	2147	TRUE	1	1.75	51	82.25	212	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Frankiales
4456598990b6bc58aeee15f041f258a7	2147	TRUE	1	6.5	21.5	73.75	216	1	1	1	1	3	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Bacillaceae;D_5__Bacillus
631226474f64e863022c47fbc00009f7	2147	TRUE	1	1	1	1	3	1	1	4	5.25	13	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
f791c7db1a78d35e84803b5e03c01965	2147	TRUE	1	4	14	42.25	70	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Pseudonocardiales;D_4__Pseudonocardiaceae;D_5__Pseudonocardia
d48d6db680d3f7b5bee737aff5727286	2144	TRUE	1	4	34.5	68.5	231	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Frankiales;D_4__Geodermatophilaceae
d292539ba659aac837e2770682eda4a2	2137	TRUE	1	3.75	22.5	55.75	101	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia;D_3__Gaiellales
8fe2c532bfdbecef39f54fede36e3458	2133	TRUE	1	4.75	24.5	56	112	1	1	1	1	3	D_0__Bacteria;D_1__Chloroflexi
ec73d0a13e9c39fecb034ef61681d3d0	2132	TRUE	1	3.75	22	65.5	99	1	1	1	1	3	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Propionibacteriales;D_4__Nocardioidaceae
47458f7a26864c20799534efcd6a865b	2130	TRUE	1	4.75	23.5	37.75	110	1	1	1	1	5	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Elsterales
79c7b38c4902075c1b5f57d507a630e8	2130	TRUE	1	3.5	26	46.75	84	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia;D_3__Solirubrobacterales;D_4__Solirubrobacteraceae;D_5__Solirubrobacter
80ab331c858f44e438a319a84298facc	2125	TRUE	1	4.75	13	24.75	79	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae
0c8bf3b2207ec839636693ee9be55df2	2122	TRUE	1	4.75	22	59.25	105	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Frankiales;D_4__Geodermatophilaceae
4ea2bee24a8209f99a0c8e60537d4e5c	2122	TRUE	1	5	17.5	56.75	99	1	1	1	1	5	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Acetobacterales;D_4__Acetobacteraceae
4403fb1a503024b1ebf12273180f7f62	2119	TRUE	1	3	17.5	30.5	64	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Rubrobacteria;D_3__Rubrobacterales;D_4__Rubrobacteriaceae;D_5__Rubrobacter
9311bffe5a78c0df3d0077d5874d6b47	2113	TRUE	1	4	15.5	32.75	63	1	1	1	1	1	D_0__Bacteria;D_1__Chloroflexi;D_2__Chloroflexia;D_3__Thermomicrobiales
d3af4a709780e098a4292f4397f55ede	2102	TRUE	18	72.75	91.5	144.25	284	1	2.5	5.5	13.25	111	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium
1234621434ce4571bb24bc90dab0530f	2092	TRUE	1	4	12.5	24	63	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Streptosporangiales;D_4__Streptosporangiaceae
35b8b34a6f148d9cca071c620f3e31fa	2086	TRUE	1	2.5	20	42	74	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia;D_3__Solirubrobacterales;D_4__Solirubrobacteraceae;D_5__Solirubrobacter
113bb511f2e84ff8c0842b9a5ca6f31a	2082	TRUE	1	4	15	24.75	98	1	1	1	1	1	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Chitinophagales;D_4__Chitinophagaceae;D_5__Segetibacter
11c76adf1b339101415f0b205cf44a39	2077	TRUE	1	3.75	13.5	30.5	65	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Pseudonocardiales;D_4__Pseudonocardiaceae;D_5__Pseudonocardia
dd9c00c47bd965236dc8c5bddd25f15e	2077	TRUE	1	3.25	11	44	92	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Propionibacteriales;D_4__Nocardioidaceae
1ce88f3f29ece47ec66e85b1b9ec5f1b	2074	TRUE	1	1	17	50.75	95	1	1	1	1	1	D_0__Bacteria;D_1__Chloroflexi
6de3541f0af38b80fbfe4fdf7e7b4dc4	2072	TRUE	2	8.25	15	23.25	718	1	1	1	1.5	10	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Micrococcaceae
a54e44b7956420fe9eb548b46b8f5626	2065	TRUE	1	4	12.5	37.25	100	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia;D_3__Solirubrobacterales
1f5eb51be02198ec44fe89e3cd6f7a23	2058	TRUE	1	2.5	17.5	38.5	83	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria
1f5025eeff0b2734a9ae6fdcb994fdc5	2058	TRUE	1	8.5	15.5	22.75	73	1	1	1	1	4	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium
5e582cb9d5f776caa8ed92f775680b66	2054	TRUE	1	3	11.5	26.75	83	1	1	1	1	3	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Microvirga
c17652ddcb9c97f0297b977358794f87	2052	TRUE	1	3	9.5	25.25	59	1	1	1	1	1	D_0__Bacteria;D_1__Chloroflexi;D_2__Ktedonobacteria

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

Table J.6 Differentially abundant fungal ASVs identified by ANCOM when comparing *Brassica napus* seed microbiomes from samples harvested in Saskatoon and Melfort during 2017.

ASV	**W	*Reject null	MF17					SK17					
			0	25	50	75	100	0	25	50	75	100	Taxonomy
ce19370a6fb2c74b9a4cc7b3733d4fb2	1288	TRUE	126	200.25	281.5	371.5	590	1	1	1	9.75	390	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Cystofilobasidiaceae;g__Cystofilobasidium;s__Cystofilobasidium_macerans
325b04fce747ff9791507c60555994eb	1287	TRUE	1	1	2.5	8.5	232	4	62.5	150	253.75	9135	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Leptosphaeriaceae
fd117529a30825ea0f76b6ca7ea0aa1e	1285	TRUE	1	352.25	438.5	546.5	934	1	1	1	1	820	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_carnescens
a9be25c1f5877c27b288acf18dd6e97f	1284	TRUE	1	1	1	7	13	1	34.5	64	137.25	12343	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Eurotiales;f__Aspergillaceae;g__Aspergillus;s__Aspergillus_ruber
45101837b8c8e9f07a31a30997d0f728	1280	TRUE	1	1	1	1	1	1	16.25	25.5	36.5	110	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Dioszegia;s__Dioszegia_xingshanensis
c1a139a96f5296a7f7be52a3b44e485d	1279	TRUE	1	157.25	194	223.5	486	1	1	1	1	279	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
1d859da4552f8aac14ac4ce577a1ce7f	1277	TRUE	1	1	1	1	5	1	11.5	26.5	53	1412	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Eurotiales;f__Aspergillaceae;g__Aspergillus
5b4b1a1f050a868547b0ff28d29504bb	1276	TRUE	1	147.75	207.5	395.25	983	1	1	1	1	447	k__Fungi;p__Ascomycota;c__Sordariomycetes
fad4e28e32b38df9f6b7216dee063980	1275	TRUE	1	1	1	11.75	74	1	35.75	71	130.75	482	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Bipolaris;s__Bipolaris_maydis
db30c1fd7cdc012398f21c386fc79558	1275	TRUE	1	1	1	1	260	1	1	103.5	157.25	283	k__Fungi;p__Basidiomycota;c__Cystobasidiomycetes;o__Cystobasidiomycetes_ord_Incertae_sedis;f__Symmetrospora;s__Symmetrospora_coprosmae
18917eb11292fdf35396480391568510	1273	TRUE	1	1	1	1	3	1	1	17.5	70.25	796	k__Fungi;p__Mucoromycota;c__Mucoromycetes;o__Mucorales;f__Rhizopodaceae;g__Rhizopus;s__Rhizopus_arrhizus
d12e2f11f2d7aea53f6ec6f355dcf8af	1271	TRUE	1	41	58	77.25	130	1	1	1	1	33	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
67f58713ac805bd9dbcd6cd68872df31	1267	TRUE	26	59.25	75	88.25	131	1	1	1	2	108	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Rhodotorula
c281d828f2aecb9ff95878688a75e829	1262	TRUE	1	1	17	22.5	37	13	29.75	55.5	79.25	162	k__Fungi;p__Basidiomycota;c__Agaricostilbomycetes;o__Agaricostilbales;f__Kondoaceae;g__Kondoa;s__Kondoa_sorbi
81dd4b112b3a91da16e30434fcd7ba1e	1261	TRUE	1	85.75	190	413	1481	30	1000.5	1803.5	2213.75	3224	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_brassicae
ecb473a0695e799edb377c51af7d0a0d	1255	TRUE	1	29.25	41.5	48.5	66	1	1	1	1	66	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Sporobolomyces;s__Sporobolomyces_roseus
1a7af98bbc390e70d1eff3383499886e	1254	TRUE	1	76.25	118	155.25	245	1	1	1	10	168	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_tephrensis
bfb189f6ade06c5933ccb7c6aa29495d	1252	TRUE	61	1528.25	2028.5	2560	3614	1	41.25	62	131.75	2700	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Sporobolomyces;s__Sporobolomyces_ruberrimus
d0f63ae4057c8d978e554cc723b10414	1249	TRUE	1891	3282.75	3761.5	4637	12769	73	223.75	274.5	378.75	5517	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
7313efe22060402792d7042e44d3282c	1240	TRUE	1	76.75	98	114.25	148	1	1	4	8.5	112	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Mrakiaceae;g__Itersonilia;s__Itersonilia_pannonica
f7082d5519038a7c8920512c97c5cde3	1235	TRUE	1	74.5	93.5	114.5	181	1	1	1	14.25	139	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleraceae;g__Bullera;s__Bullera_crocea
2b9d36de3f3124e8d011dc13b3770d3b	1223	TRUE	1	61	113	172.75	577	1	1	1	1	416	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Didymellaceae
4eac723a6a9e8217f28c2ab549602833	1218	TRUE	1	120.5	152.5	183.25	274	1	1	1	39.25	275	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
f033b275ef5d3e2480a136eef0e4df31	1216	TRUE	1	1	37.5	55.25	1737	1	1	1	1	1	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Sporobolomyces;s__Sporobolomyces_roseus
8b41e27a4be11eb0b559db7ded7dd91b	1216	TRUE	539	2988.5	3449	4279	7015	143	332.5	417.5	611	5651	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
f0dd7217363efbda3a3d23482db0197b	1214	TRUE	1	22.5	57.5	176.5	369	1	1	1	7	174	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Cordycipitaceae;g__Lecanicillium;s__Lecanicillium_muscarium
7999061f04b0be558c7c285486732789	1214	TRUE	1	15	22	35.5	144	1	1	1	1	44	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Dioszegia;s__Dioszegia_fristingensis
a7b84bdccfd4af32fcc14f18b818e6aa	1210	TRUE	1	113	138	168.75	334	1	1	16.5	28.25	152	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_tephrensis
75a9b73903a3053226dff54454c05cd9	1202	TRUE	1	16.75	27	45.75	164	1	1	1	1	168	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Erysiphales;f__Erysiphaceae;g__Blumeria;s__Blumeria_graminis
8a5726aa2c7721a33c6cf40a51a920b5	1202	TRUE	1	23	37	47.5	95	1	1	1	11.25	50	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
628c5c4057f4a14adcbf8b7d11e4b742	1199	TRUE	1	1	109	130.5	170	1	1	1	1	199	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales
d8433719787 3c342e907796 1140b14b6	1182	TRUE	1	13.75	25.5	40.5	63	1	1	1	1	62	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Rhodotorula;s__Rhodotorula_graminis
e1223cae4a476801fa9f1945123a34a0	1178	TRUE	1	130.5	171.5	220.75	384	1	1	1	71.75	421	k__Fungi;p__Basidiomycota;c__Cystobasidiomycetes;o__Cystobasidiomycetes_ord_Incertae_sedis;f__Symmetrospraceae;g__Symmetrospora;s__Symmetrospora_coprosmae
4ba2d495b2c390f587b7bb73ddd34eed	1176	TRUE	130	228.5	297	388.25	2601	1	35.75	66.5	110	489	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Filobasidiales;f__Filobasidiaceae;g__Filobasidium;s__Filobasidium_chernovii

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

Table J.7 Differentially abundant fungal ASVs identified by ANCOM when comparing *Brassica napus* seed microbiomes from samples harvested in Saskatoon and Scott during 2017.

ASV	**W	*Reject null	SC17					SK17					
			0	25	50	75	100	0	25	50	75	100	Taxonomy
9d6eddf36e03d4d20c1ad1cdfa130688	826	TRUE	87	283.5	757	1638.25	8122	1	1	1	1.75	20	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Trichothecium;s__Trichothecium_roseum
3aa767c6a95d6eaa108796a59afea57e	826	TRUE	17	87.25	152	429.25	15781	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Trichothecium;s__Trichothecium_roseum
79c42a151d54851ad3f64ebd6b5ad1bc	825	TRUE	138	254.75	371	462.25	1354	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Didymellaceae
25e062f8848d484726ecfbabca48d532	819	TRUE	30	54.75	63.5	115.5	450	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_subcucurbitae
67f58713ac805bd9dbcd6cd68872df31	818	TRUE	35	86.75	122.5	188.25	348	1	1	1	2	108	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Rhodotorula
e31354c24db1fbae54d7811869386645	817	TRUE	86	152	244.5	377	721	1	1	9.5	12	27	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Cystofilobasidiaceae;g__Cystofilobasidium;s__Cystofilobasidium_macerans
b0f188f5375515db6ff8bdafc2095eeb	817	TRUE	663	1327.5	1965.5	2951.25	7609	1	28.5	52.5	143.5	258	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Cystofilobasidiaceae;g__Cystofilobasidium;s__Cystofilobasidium_macerans
fad4e28e32b38df9f6b7216dee063980	815	TRUE	1	1	1	1	1	1	35.75	71	130.75	482	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Bipolaris;s__Bipolaris_maydis
b2f69980afba9361df100caebe97fb82	815	TRUE	68	308	519	645.75	1144	1	23.25	39	69	142	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Holtermanniales;f__Holtermanniales_fam_Incertae_sedis;g__Holtermanniella;s__Holtermanniella_takashimae
63d3734f6c084466d644f3dd20ebca8b	814	TRUE	1	1	1	1	1	1	20.5	41.5	70	309	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae
ce19370a6fb2c74b9a4cc7b3733d4fb2	814	TRUE	11	43.25	85.5	124	354	1	1	1	9.75	390	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Cystofilobasidiaceae;g__Cystofilobasidium;s__Cystofilobasidium_macerans
79ac0619fd94699ce7380fa269effaa2	812	TRUE	1	1	69.5	109.5	258	1	1	1	1	1	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Filobasidiales;f__Filobasidiaceae;g__Filobasidium;s__Filobasidium_magnum
bdb2e5d386b79e8cf6d5a4e9dd1c064d	812	TRUE	29	135.25	247.5	295	513	1	1	25	47.25	155	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Holtermanniales;f__Holtermanniales_fam_Incertae_sedis;g__Holtermanniella;s__Holtermanniella_takashimae
d9f58908e54fea3618f22844d0c556a6	809	TRUE	1	1	1	1	31	1	26.25	54.5	102.5	1627	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_oboviodea
3bdc8db1b460584d2e8904d34a3fc753	807	TRUE	1	11	21	44	648	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium;s__Fusarium_poae
d65c59c35bde2c2e7ee76e74e1a8f078	807	TRUE	1	1	23	40.5	98	84	134.75	189.5	235.75	480	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_carnescens
5e601f1cbc8b00d3a58e7d6d1f39baae	806	TRUE	1	1	11.5	22.25	169	32	102.25	124.5	250.25	497	k__Fungi;p__Ascomycota;c__Sordariomycetes
9a951f3d89952c10e7436fe4e38d8f89	802	TRUE	1	12	23	66.5	205	1	1	1	1.5	14	k__Fungi
c57ba71f5c971de6c317692acf15e6e3	802	TRUE	1	24.5	35	55.25	361	1	1	1	1	105	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
c1a139a96f5296a7f7be52a3b44e485d	802	TRUE	1	48.75	84.5	121.25	225	1	1	1	1	279	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
c281d828f2aecb9ff95878688a75e829	792	TRUE	1	1	1	15	40	13	29.75	55.5	79.25	162	k__Fungi;p__Basidiomycota;c__Agaricostilbomycetes;o__Agaricostilbales;f__Kondoaceae;g__Kondoa;s__Kondoa_sorbi
ba095f1690a3b11630aace84c78cb556	785	TRUE	1	1	1	1	128	1	49.75	87.5	106.5	221	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Mycosphaerellaceae;g__Mycosphaerella;s__Mycosphaerella_tassiana
509a5fa82bf4bf431a401ee2db5bbc8c	785	TRUE	1	1	6.5	15.5	135	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Microascales;f__Microascaceae;g__Microascus;s__Microascus_brevicaulis
db30c1fd7cdc012398f21c386fc79558	783	TRUE	1	1	1	1	45	1	1	103.5	157.25	283	k__Fungi;p__Basidiomycota;c__Cystobasidiomycetes;o__Cystobasidiomycetes_ord_Incertae_sedis;f__Symmetrospraceae;g__Symmetrospora;s__Symmetrospora_coprosmae
18917eb11292fdf35396480391568510	780	TRUE	1	1	1	1	1	1	1	17.5	70.25	796	k__Fungi;p__Mucoromycota;c__Mucoromycetes;o__Mucorales;f__Rhizopodaceae;g__Rhizopus;s__Rhizopus_arrhizus
5a5a657103448a51240c20591cdb61b4	778	TRUE	1	5.5	10	16	50	1	40.75	68.5	148	678	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Sarocladium;s__Sarocladium_strictum
325b04fce747ff9791507c60555994eb	773	TRUE	1	1	8.5	29.75	140	4	62.5	150	253.75	9135	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Leptosphaeriaceae
1d859da4552f8aac14ac4ce577a1ce7f	773	TRUE	1	1	1	1	16	1	11.5	26.5	53	1412	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Eurotiales;f__Aspergillaceae;g__Aspergillus
3836f16615f65eff08ee4dbf92177a5b	769	TRUE	139	310.5	412.5	580.75	964	25	86.5	132.5	210.75	590	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Paradendryphiella;s__Paradendryphiella_arenariae
45101837b8c8e9f07a31a30997d0f728	761	TRUE	1	1	1	2	13	1	16.25	25.5	36.5	110	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Dioszegia;s__Dioszegia_xingshanensis
8eca0e63a95e19e37ab7f4232fe87e1c	759	TRUE	1	1	6.5	13.25	30	1	1	1	1	1	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Leucosporidiales;f__Leucosporidiaceae;g__Leucosporidium;s__Leucosporidium_fragarium
b868159fd517bf07e6182e1c608948a8	753	TRUE	1	1	5.5	9.25	18	1	16.75	22	47.75	92	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Dothideales;f__Aureobasidiaceae;g__Aureobasidium;s__Aureobasidium_pullulans
dd9a6a4f9596f0541b4001eb9d6f4fbf	749	TRUE	1	1	1	9.25	34	1	16.25	36.5	59.25	298	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
ff1764a27bc90328a6d970f9733b58c6	747	TRUE	1	1	5	16	27	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Didymellaceae;g__Didymella;s__Didymella_exigua
81dd4b112b3a91da16e30434fcd7ba1e	745	TRUE	43	91.5	145	252.75	684	30	1000.5	1803.5	2213.75	3224	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_brassicae

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

Table J.8 Differentially abundant fungal ASVs identified by ANCOM when comparing *Brassica napus* seed microbiomes from samples harvested in Melfort and Scott during 2017.

ASV	**W	*Reject null	MF17					SC17					Taxonomy
			0	25	50	75	100	0	25	50	75	100	
fd117529a30825ea0f76b6ca7ea0aa1e	1049	TRUE	1	352.25	438.5	546.5	934	1	1	1	1	1	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_carnescens
5b4b1a1f050a868547b0ff28d29504bb	1049	TRUE	1	147.75	207.5	395.25	983	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes
25e062f8848d484726ecfbabca48d532	1049	TRUE	1	1	1	1	1	30	54.75	63.5	115.5	450	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_subcucurbitae
9d6eddf36e03d4d20c1ad1cdfa130688	1048	TRUE	1	1	1	1	17	87	283.5	757	1638.25	8122	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Trichothecium;s__Trichothecium_roseum
3aa767c6a95d6eaa108796a59afea57e	1048	TRUE	1	1	1	1	1	17	87.25	152	429.25	15781	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Trichothecium;s__Trichothecium_roseum
79c42a151d54851ad3f64ebd6b5ad1bc	1047	TRUE	1	1	1	1	1	138	254.75	371	462.25	1354	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Didymellaceae
9a951f3d89952c10e7436fe4e38d8f89	1043	TRUE	1	1	1	1	1	1	12	23	66.5	205	k__Fungi
1a7af98bbc390e70d1eff3383499886e	1036	TRUE	1	76.25	118	155.25	245	1	1	1	1	14	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_tephrensis
63d3734f6c084466d644f3dd20ebca8b	1035	TRUE	1	51.25	87	125.5	233	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae
3bdc8db1b460584d2e8904d34a3fc753	1035	TRUE	1	1	1	1	1	1	11	21	44	648	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium;s__Fusarium_poae
d12e2f11f2d7aea53f6ec6f355dcf8af	1028	TRUE	1	41	58	77.25	130	1	1	1	1	1	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
79ac0619fd94699ce7380fa269effaa2	1027	TRUE	1	1	1	1	1	1	1	69.5	109.5	258	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Filobasidiales;f__Filobasidiaceae;g__Filobasidium;s__Filobasidium_magnum
f7082d5519038a7c8920512c97c5cde3	1025	TRUE	1	74.5	93.5	114.5	181	1	1	1	1	15	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleraceae;g__Bullera;s__Bullera_crocea
f0dd7217363efbda3a3d23482db0197b	1024	TRUE	1	22.5	57.5	176.5	369	1	1	1	1	3	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Cordycipitaceae;g__Lecanicillium;s__Lecanicillium_muscarium
4eac723a6a9e8217f28c2ab549602833	1023	TRUE	1	120.5	152.5	183.25	274	1	1	1	2.5	24	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
5e557a68e85ee03eaebc52062dcdff98	1020	TRUE	1	26	34	51.5	108	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Xylariales;f__Microdochiaceae;g__Microdochium;s__Microdochium_paspali
bfb189f6ade06c5933ccb7c6aa29495d	1017	TRUE	61	1528.25	2028.5	2560	3614	1	30.25	70.5	92.5	184	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Sporobolomyces;s__Sporobolomyces_ruberrimus
ecb473a0695e799edb377c51af7d0a0d	1016	TRUE	1	29.25	41.5	48.5	66	1	1	1	1	1	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Sporobolomyces;s__Sporobolomyces_roseus
dd9a6a4f9596f0541b4001eb9d6f4fbf	1015	TRUE	1	120	155.5	201.25	275	1	1	1	9.25	34	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
c57ba71f5c971de6c317692acf15e6e3	1014	TRUE	1	1	1	5.25	70	1	24.5	35	55.25	361	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium
5d03e3ed7872d20eb91be46351a57b05	1012	TRUE	1	1	1	41.25	100	1	40	54.5	59.5	147	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_carnescens
5a5a657103448a51240c20591cdb61b4	1008	TRUE	1	351.25	464	648.25	783	1	5.5	10	16	50	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Hypocreales_fam_Incertae_sedis;g__Sarocladium;s__Sarocladium_strictum
b0f188f5375515db6ff8bdafc2095eeb	1007	TRUE	97	168.5	210.5	315.75	7573	663	1327.5	1965.5	2951.25	7609	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Cystofilobasidiaceae;g__Cystofilobasidium;s__Cystofilobasidium_macerans
509a5fa82bf4bf431a401ee2db5bbc8c	1006	TRUE	1	1	1	1	1	1	1	6.5	15.5	135	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Microascales;f__Microascaceae;g__Microascus;s__Microascus_brevicaulis
5d8005b324745ac4439e6831a5c2135a	1003	TRUE	1	946	1231.5	1498.75	2349	1	18.5	29.5	52.25	87	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
8a5726aa2c7721a33c6cf40a51a920b5	1002	TRUE	1	23	37	47.5	95	1	1	1	1	11	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
628c5c4057f4a14adcbf8b7d11e4b742	1002	TRUE	1	1	109	130.5	170	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales
1ac7001628cd5926185b585586fb058d	994	TRUE	1	10.5	20	28.75	59	1	1	1	1	1	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Rhynchogastremataceae;g__Papiliotrema;s__Papiliotrema_flavescens
498f1bed8eaa99209cd63956ba4ad21a	994	TRUE	1	19	29.5	48	66	1	1	1	3	8	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Rhynchogastremataceae;g__Papiliotrema;s__Papiliotrema_aurea
b868159fd517bf07e6182e1c608948a8	994	TRUE	18	54.75	62.5	79.5	106	1	1	5.5	9.25	18	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Dothideales;f__Aureobasidiaceae;g__Aureobasidium;s__Aureobasidium_pullulans
568dbc1d0aad7dde78fe451cee3261c3	991	TRUE	1	31.75	37	46.25	83	1	1	1	1	34	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Phaeosphaeriaceae;g__Parastagonospora;s__Parastagonospora_poae
a7b84bdccfd4af32fcc14f18b818e6aa	987	TRUE	1	113	138	168.75	334	1	1	15.5	22.25	36	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_tephrensis
8eca0e63a95e19e37ab7f4232fe87e1c	985	TRUE	1	1	1	1	1	1	1	6.5	13.25	30	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Leucosporidiales;f__Leucosporidiaceae;g__Leucosporidium;s__Leucosporidium_fragarium
918460ba365dfd82c6e2097d3be06425	980	TRUE	1	25.75	33.5	43	69	1	1	1	2.5	17	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Dioszegia;s__Dioszegia_butyracea
e31354c24db1fbae54d7811869386645	979	TRUE	1	11	18	47.5	6540	86	152	244.5	377	721	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Cystofilobasidiaceae;g__Cystofilobasidium;s__Cystofilobasidium_macerans
386e54fe292c2f421b3f407434f6d9b8	978	TRUE	1	1	1	1	28	1	1	6	13.25	220	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Glomerellales;f__Plectosphaerellaceae;g__Plectosphaerella;s__Plectosphaerella_oratosquillae
ba095f1690a3b11630aace84c78cb556	977	TRUE	1	95	119	131.75	245	1	1	1	1	128	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Mycosphaerellaceae;g__Mycosphaerella;s__Mycosphaerella_tassiana
ff1764a27bc90328a6d970f9733b58c6	973	TRUE	1	1	1	1	1	1	1	5	16	27	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Didymellaceae;g__Didymella;s__Didymella_exigua
b2f69980afba9361df100caebe97fb82	970	TRUE	38	81.75	108	140.25	1129	68	308	519	645.75	1144	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Holtermanniales;f__Holtermanniales_fam_Incertae_sedis;g__Holtermanniella;s__Holtermanniella_takashimae
e1f579c16ab012b62ecee5df562e21d9	968	TRUE	41	164.5	191.5	226	377	1	13	25	36.5	92	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Mycosphaerellaceae;g__Mycosphaerella;s__Mycosphaerella_tassiana
4cd69b20518d71a7465973475 39ac21c	965	TRUE	1	1	1	1	1	1	1	4	10	14	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Mycosphaerellaceae;g__Septoria;s__Septoria_cretae
f033b275ef5d3e2480a136eef0e4df31	965	TRUE	1	1	37.5	55.25	1737	1	1	1	1	1	k__Fungi;p__Basidiomycota;c__Microbotryomycetes;o__Sporidiobolales;f__Sporidiobolaceae;g__Sporobolomyces;s__Sporobolomyces_roseus
fe7a923bf449b815463941cab35d72bc	962	TRUE	1	1	1	1	1	1	1	2.5	15	31	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Mycosphaerellaceae;g__Mycosphaerella;s__Mycosphaerella_tassiana
83cbd2de93b9aa5206ed87138fc957a1	961	TRUE	1	28.75	45.5	54.75	86	1	1	4.5	8	21	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Hannaella;s__Hannaella_coprosmae
ea0cca30ba680cf084edc628166a4916	960	TRUE	1	1	12	19.25	38	1	1	1	1	1	k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Cordycipitaceae;g__Beauveria;s__Beauveria_bassiana
75a9b73903a3053226dff54454c05cd9	960	TRUE	1	16.75	27	45.75	164	1	1	1	5.25	17	k__Fungi;p__Ascomycota;c__Leotiomycetes;o__Erysiphales;f__Erysiphaceae;g__Blumeria;s__Blumeria_graminis
d0f63ae4057c8d978e554cc723b10414	958	TRUE	1891	3282.75	3761.5	4637	12769	267	482.75	842	1128.25	1873	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
31ce3d29b4e52f21c1e087c83552375b	955	TRUE	1	1	40	48.5	90	1	1	1	1	13	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_tephrensis
bb7cb4bfb8fc6e6dc4d1cf1f1e9f68d4	950	TRUE	1	1	1	1	1	1	1	4	17.75	63	k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Eurotiales;f__Aspergillaceae;g__Penicillium;s__Penicillium_polonicum
d65c59c35bde2c2e7ee76e74e1a8f078	948	TRUE	1	188.75	241	290.75	482	1	1	1	23	40.5	98	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_carnescens
093954a6eb2a2cfda685ff81f50ce8ba	946	TRUE	1	13.75	29.5	46.25	184	1	1	1	5.75	28	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Didymosphaeriaceae;g__Pseudopithomyces
2ed87eda45a255b67386d43c4840cb25	943	TRUE	1	1	35.5	50	72	1	1	1	1	1	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_tephrensis
8b41e27a4be11eb0b559db7ded7dd91b	942	TRUE	539	2988.5	3449	4279	7015	227	490.75	825.5	977.75	3355	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
96f24862bb0534f504648d677d32cba7	935	TRUE	27	438	579.5	745.25	948	4	22	104	156.25	393	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Dioszegia;s__Dioszegia_hungarica
fe07aa60f9231191add4319a41b448cb	934	TRUE	1	1	62.5	78	117	1	1	1	1	1	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Tremellales;f__Bulleribasidiaceae;g__Vishniacozyma;s__Vishniacozyma_victoriae
d9f58908e54fea3618f22844d0c556a6	923	TRUE	1	1	17	101.25	675	1	1	1	1	31	k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Pleosporaceae;g__Alternaria;s__Alternaria_obovoidea
ce19370a6fb2c74b9a4cc7b3733d4fb2	914	TRUE	126	200.25	281.5	371.5	590	11	43.25	85.5	124	354	k__Fungi;p__Basidiomycota;c__Tremellomycetes;o__Cystofilobasidiales;f__Cystofilobasidiaceae;g__Cystofilobasidium;s__Cystofilobasidium_macerans

* Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

APPENDIX K: α- and β-Diversity estimators of soil bacterial communities from Saskatoon and Scott locations.

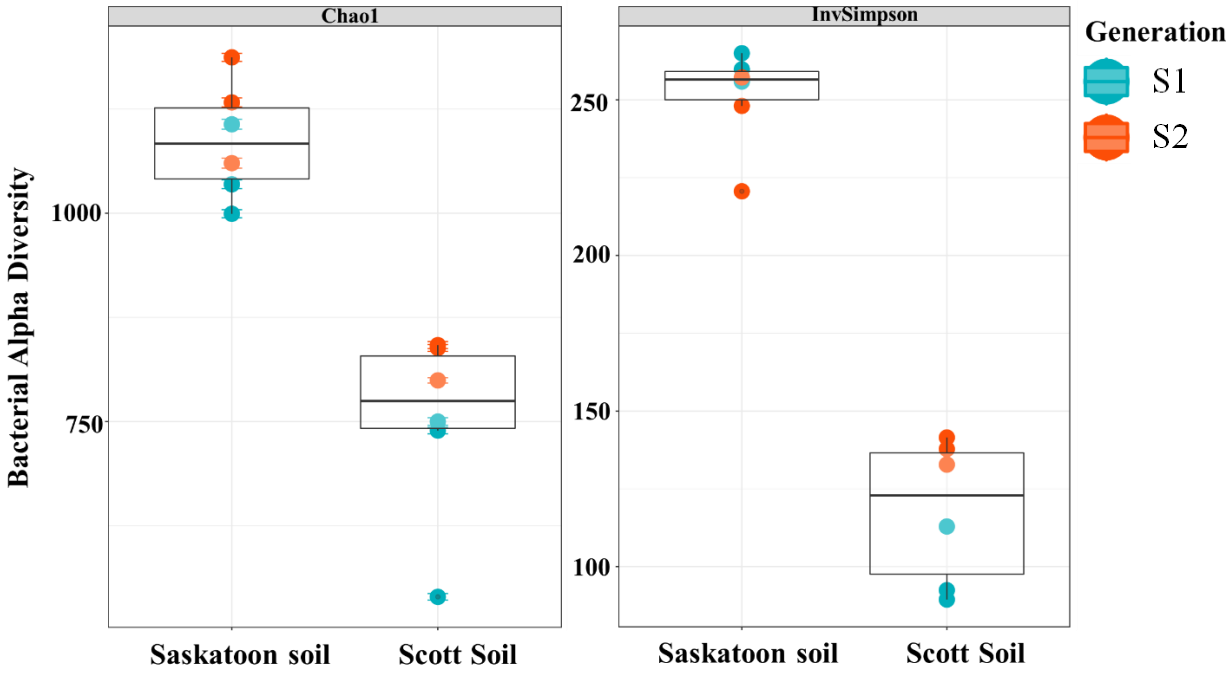

Figure K.1 Box plots depicting estimated richness (Chao1 index) and diversity (Inverse Simpson's index) of bacterial communities (16S rRNA gene) associated with bulk soils collected from Saskatoon and Scott locations in Saskatchewan, Canada. S1 represents the soil collected from the field and used to yield first generation seeds (G1). S2 represents the soil collected from the field and used to yield second generation seeds (G2). Soils used to grow both generations were from the same location in the same field site.

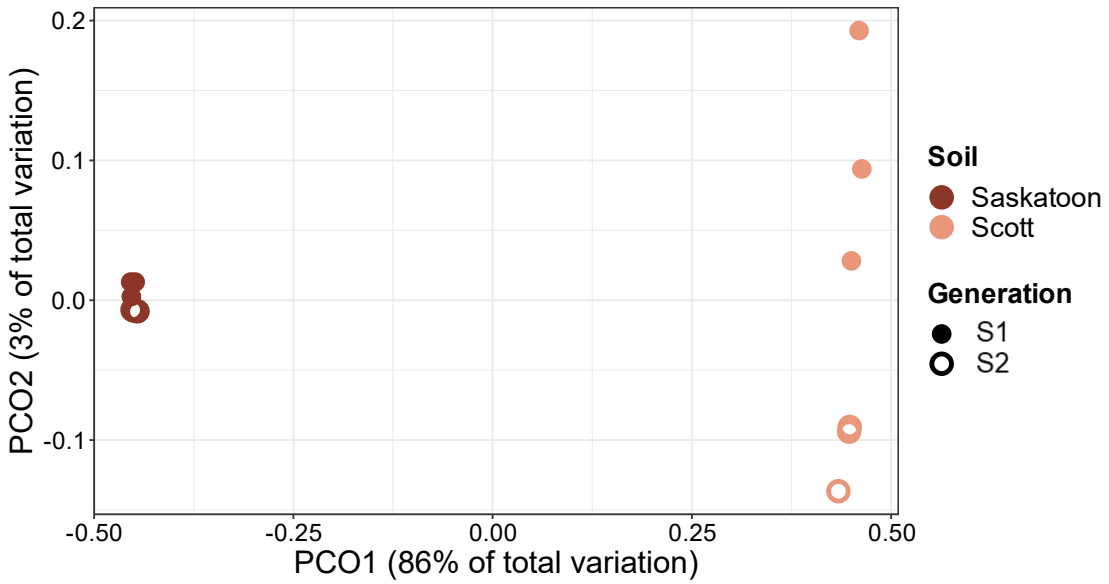

Figure K.2 Principal coordinate analysis (PCoA) based on Bray-Curtis index of bacterial communities (16S rRNA gene) associated with bulk soils collected from Saskatoon and Scott locations in Saskatchewan, Canada. S1 represents the soil collected from the field and used to yield first generation seeds (G1). S2 represents the soil collected from the field and used to yield second generation seeds (G2). Soils used to grow both generations were from the same location in the same field site.

APPENDIX L: Relative abundance of bacterial phyla in Saskatoon and Scott bulk soils.

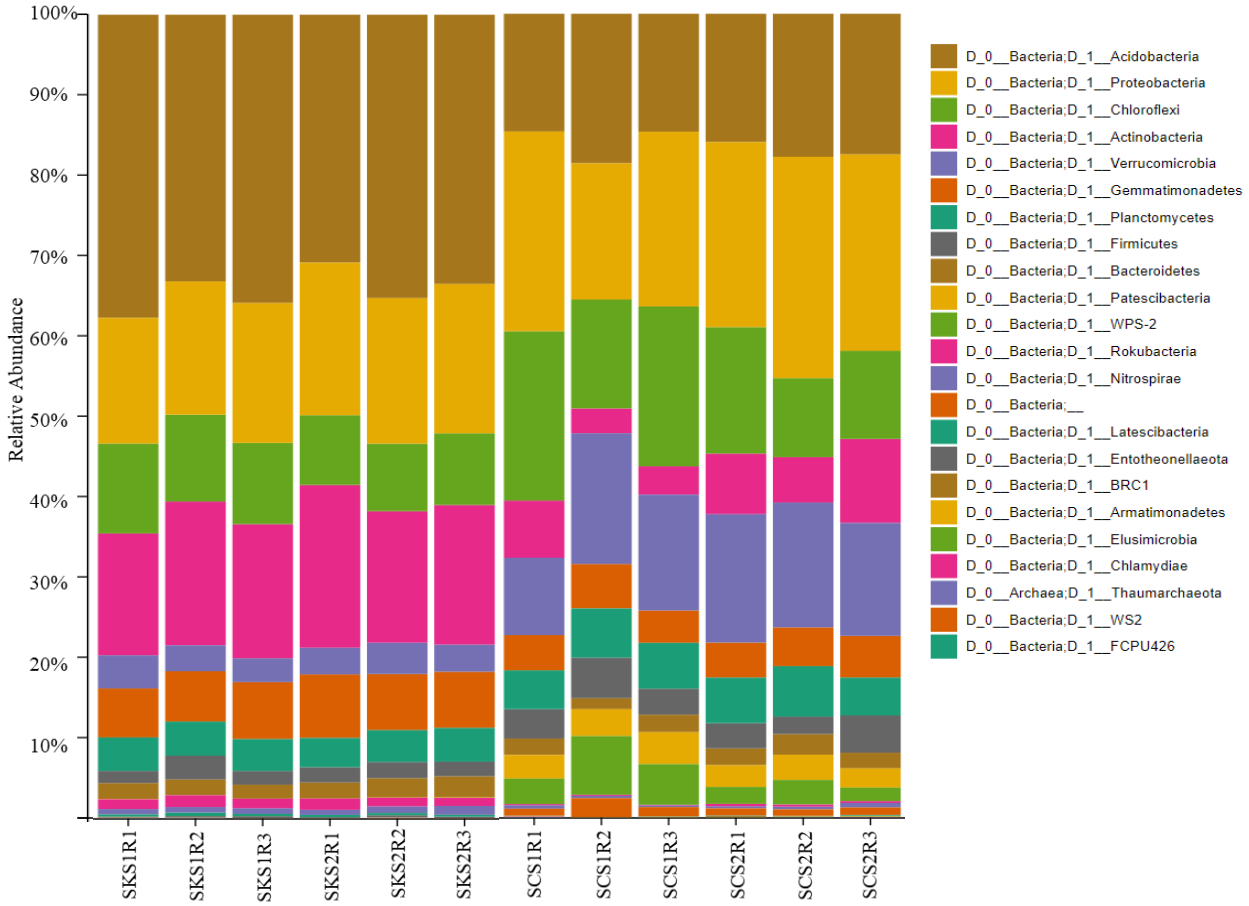

Figure L.1 Relative abundance of the dominant bacterial phyla associated with Saskatoon and Scott bulk soils. SK= Saskatoon, SC= Scott, S1= soil collected from the field and used to yield first generation seeds, S2= soil collected from the field and used to yield second generation seeds, R1-R3 = Replicate.

Table L.1 Differentially abundant bacterial ASVs in Saskatoon and Scott bulk soils, identified by pairwise analysis of microbiome composition (ANCOM).

	**W	*Reject null	Saskatoon					Scott					Taxonomy
Percentile			0	25	50	75	100	0	25	50	75	100	
e5ddfb6474cdd68b300b69ad21f1d943	2642	TRUE	1	1	1	1	1	144	184	284	591.75	945	D_0__Bacteria;D_1__Chloroflexi;D_2__Ktedonobacteria;D_3__Ktedonobacterales;D_4__Ktedonobacteraceae
c06ea72cd28b4d0acbcaad9c73b5e659	2641	TRUE	1	1	1	1	3	156	418.25	444.5	521.75	569	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Xanthobacteraceae;D_5__Pseudolabrys
5e43f2d8be71399fc8d6736754cb42ac	2638	TRUE	77	90.75	104	127	150	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Azospirillales;D_4__Azospirillaceae;D_5__Skermanella
7bff03cb7e71da08a83bce6eb2813b14	2638	TRUE	63	83.75	96	118.75	146	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Rubrobacteria;D_3__Rubrobacterales;D_4__Rubrobacteriaceae;D_5__Rubrobacter
ce05f58164e61517198f7ab0e3304cb5	2637	TRUE	112	132.5	158	176	253	1	1	1	1	3	D_0__Bacteria;D_1__Acidobacteria;D_2__Blastocatellia (Subgroup 4);D_3__Blastocatellales;D_4__Blastocatellaceae
1f5eb51be02198ec44fe89e3cd6f7a23	2637	TRUE	72	84.25	93	103.25	111	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria
013e849796b4523ddf4b934766d413ad	2636	TRUE	1	1	1	1	1	101	163.25	215.5	223.5	238	D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Acidobacteriales;D_4__Koribacteraceae;D_5__Candidatus Koribacter
9fdf4417b89da215ff08562768b5922b	2636	TRUE	71	78.25	80	109.5	122	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria
a7a777eb91bb79b5a2217478056fa08f	2633	TRUE	51	80.5	103.5	122	153	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria;D_2__Blastocatellia (Subgroup 4);D_3__Pyrinomonadales;D_4__Pyrinomonadaceae
1907375e22248cebb8a1d093b0b76334	2632	TRUE	58	66.25	85.5	109.25	126	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria
b3a54fc1b66cedf5be3fd65507ba99ae	2630	TRUE	1	1	1	1	1	62	149.5	177.5	222.75	285	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria
fdaca5035b3eb8725462b7dad3783cf2	2627	TRUE	1	1	1	1	1	85	119.25	166	218	440	D_0__Bacteria;D_1__Chloroflexi
42b3b931bc5808b6eb2f6ebdb799fe51	2625	TRUE	51	66	82.5	116.25	138	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria;D_2__Blastocatellia (Subgroup 4);D_3__Pyrinomonadales;D_4__Pyrinomonadaceae
4e637b0d7dabc838190d58cc8cbba39b	2622	TRUE	51	56.75	60	71.5	85	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria
220db2df1b1dd5ac031ffb1c9db32766	2618	TRUE	42	46.25	51.5	58.25	75	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria
352e42d5dc9f3f10b0efe9fc701e21af	2612	TRUE	1	1	1	1	1	70	112.75	130.5	149	175	D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Acidobacteriales
780e625c33b476b0741975c6f3c04030	2600	TRUE	1	1	1	1	1	64	79.75	112.5	122.75	139	D_0__Bacteria;D_1__Acidobacteria;D_2__Blastocatellia (Subgroup 4);D_3__Blastocatellales;D_4__Blastocatellaceae
49d37c2625a68f41f4357ef4267d98f4	2596	TRUE	1	1	1	1	1	46	102.25	113.5	125.5	158	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales
c4593f2a6b44a0c31e0e74f4dc1d3201	2594	TRUE	41	46.75	49	53.5	56	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria
aa430bb414c73584fb067867bd4e95bb	2594	TRUE	35	39.5	48	63.25	75	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria;D_2__Blastocatellia (Subgroup 4);D_3__Pyrinomonadales;D_4__Pyrinomonadaceae
f05e4252d1616a13033c570ee40e8a3f	2591	TRUE	1	1	1	1	1	57	84	94.5	146.25	211	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria
99233347bda34be547c42314d9c90649	2587	TRUE	1	1	1	1	1	47	81.25	99.5	130.5	180	D_0__Bacteria;D_1__Chloroflexi;D_2__Ktedonobacteria;D_3__Ktedonobacterales;D_4__Ktedonobacteraceae
6de00c50deee8f4bb5bce2aad90264e8	2582	TRUE	1	1	1	1	1	59	74	81	92.5	105	D_0__Bacteria;D_1__Gemmatimonadetes;D_2__Gemmatimonadetes;D_3__Gemmatimonadales;D_4__Gemmatimonadaceae;D_5__Gemmatimonas
d894182fbfd26da5669e2330c3400f99	2575	TRUE	1	1	1	1	1	37	73.75	80.5	95.5	127	D_0__Bacteria;D_1__Patescibacteria;D_2__Parcubacteria;D_3__Candidatus Adlerbacteria
2a9054ad60367a6de9e9dfa2a6531394	2574	TRUE	36	39.25	47.5	56.5	64	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia;D_3__Gaiellales
cc9b2229a686027ab9d675d21c9e1ee5	2572	TRUE	1	1	1	1	1	55	79.25	104.5	111	119	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Micropepsales;D_4__Micropepsaceae
b6ecbb10318cfd63a6e83e8944ef3301	2568	TRUE	1	1	1	1	1	70	73	96	122.75	197	D_0__Bacteria
afc77916972cb0e347d3f563e775f2a6	2567	TRUE	30	37.5	39.5	40.75	46	1	1	1	1	1	D_0__Bacteria;D_1__Planctomycetes;D_2__Phycisphaerae;D_3__Tepidisphaerales
f1d6c68964f9bd9219be533b51359d70	2563	TRUE	33	42.75	46.5	53.25	61	1	1	1	1	1	D_0__Bacteria;D_1__Chloroflexi;D_2__Chloroflexia;D_3__Thermomicrobiales
ba81cbfcc6c899dc09762e638cb6324d	2557	TRUE	28	35	44.5	45.75	58	1	1	1	1	1	D_0__Bacteria;D_1__Gemmatimonadetes;D_2__Gemmatimonadetes;D_3__Gemmatimonadales;D_4__Gemmatimonadaceae
f27fe2af5abe3dbcf79b374ddb063a8a	2556	TRUE	1	1	1	1	1	43	62.75	71.5	72.75	103	D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Acidobacteriales;D_4__uncultured
cb504c763c2be33a34cf50b0e3b44670	2548	TRUE	1	1	1	1	1	38	55.75	61.5	63.5	87	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Micropepsales;D_4__Micropepsaceae
173b6c006c32e0a520107008dc44cf16	2537	TRUE	1	1	1	1	1	44	57.75	63.5	66.25	72	D_0__Bacteria;D_1__Gemmatimonadetes;D_2__Gemmatimonadetes;D_3__Gemmatimonadales;D_4__Gemmatimonadaceae;D_5__Gemmatimonas
c71620130de631426987c6844fbc17f1	2537	TRUE	1	1	1	1	1	43	55	62	66	69	D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Acidobacteriales;D_4__Acidobacteriaceae (Subgroup 1);D_5__Granulicella
9b7d2a468bb85840d258f8ab732ebf0a	2533	TRUE	1	1	1	1	1	43	49.5	68.5	80.75	105	D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Acidobacteriales;D_4__uncultured
8c0c5dcceceac4ab4125d1364c743247	2530	TRUE	1	1	1	1	1	49	69.25	84	107.75	195	D_0__Bacteria;D_1__Chloroflexi;D_2__Ktedonobacteria;D_3__Ktedonobacterales;D_4__Ktedonobacteraceae
e3301471f7a43035e957f3515bc163a7	2527	TRUE	30	33	36	37.5	41	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Myxococcales
a79ebe3296a619b2979497dea68f1bf9	2524	TRUE	1	1	1	1	1	37	47.5	57.5	67.5	73	D_0__Bacteria;D_1__Gemmatimonadetes;D_2__Gemmatimonadetes;D_3__Gemmatimonadales;D_4__Gemmatimonadaceae;D_5__Gemmatimonas
0b3bb5cb72691b020254b4a772c40ec7	2513	TRUE	28	32.25	43	44.75	59	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria
9657d52acca51e701e7a3b7cedcfcc12	2508	TRUE	24	34.25	37.5	40.75	42	1	1	1	1	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Azospirillales;D_4__Azospirillaceae
b3c74f006ad15a2f3f32b47f221c3389	2489	TRUE	1	1	1	1	1	44	46.25	48.5	59	74	D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Acidobacteriales;D_4__Acidobacteriaceae (Subgroup 1)
d615674525cd828223413e0d430ab4fb	2479	TRUE	1	1	1	1	1	40	54.25	86.5	145.75	181	D_0__Bacteria;D_1__Chloroflexi;D_2__Ktedonobacteria;D_3__Ktedonobacterales;D_4__Ktedonobacteraceae
cdaabfd8088930c4a268b4b7b599978d	2479	TRUE	26	28	31	37.75	55	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria;D_2__Blastocatellia (Subgroup 4);D_3__Pyrinomonadales;D_4__Pyrinomonadaceae
c468da0859abf6be7adc7ae4e1a0b1fa	2473	TRUE	1	1	1	1	1	27	40.5	49.5	53.25	56	D_0__Bacteria;D_1__Gemmatimonadetes;D_2__Gemmatimonadetes;D_3__Gemmatimonadales;D_4__Gemmatimonadaceae
9b610781d0c41d47790df4e1a350b672	2470	TRUE	21	30.5	36	43	63	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria
e4f830aedf7d95e0cc3ff4b1faa2c597	2469	TRUE	1	1	1	1	1	28	49.25	57.5	77	135	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Frankiales;D_4__Acidothermaceae;D_5__Acidothermus
3185f61b4be171aa11addec0e61d6a6f	2450	TRUE	1	1	1	1	1	37	43.5	45	46.5	82	D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Chthoniobacterales;D_4__Chthoniobacteraceae
fa0aa6a7cde6285eea1d9415cd09d5ff	2442	TRUE	1	1	1	1	1	30	36	46	61.25	67	D_0__Bacteria;D_1__Chloroflexi;D_2__Ktedonobacteria;D_3__Ktedonobacterales;D_4__Ktedonobacteraceae
2bc3a77e4035210ea9cb46f6c5423326	2434	TRUE	1	1	1	1	1	36	49	63.5	69	85	D_0__Bacteria;D_1__Planctomycetes;D_2__Phycisphaerae;D_3__Tepidisphaerales
0f91fa24341ac18ccceb7ffe9f487d66	2433	TRUE	22	26	38.5	45	57	1	1	1	1	1	D_0__Bacteria;D_1__Actinobacteria;D_2__Rubrobacteria;D_3__Rubrobacterales;D_4__Rubrobacteriaceae;D_5__Rubrobacter
ddb55d57211bc0b63bd8597003dbd7dd	2432	TRUE	1	1	1	1	1	37	42.5	67.5	134.5	188	D_0__Bacteria;D_1__Chloroflexi;D_2__Ktedonobacteria;D_3__Ktedonobacterales;D_4__Ktedonobacteraceae
727fb1158f479567348fb8a5581347d5	2426	TRUE	1	1	1	1	1	21	35.25	40	41.75	53	D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Acidobacteriales;D_4__Acidobacteriaceae (Subgroup 1);D_5__Occallatibacter
e805d9ba5cbb6baa4d7cdc5b609908bd	2422	TRUE	1	1	1	1	1	54	66	107.5	235.25	325	D_0__Bacteria;D_1__Firmicutes
929995b67e83e10b35fb2a009a34808a	2421	TRUE	1	1	1	1	1	22	32.5	45	56.75	62	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Elsterales
3e006a0129095d17e65abbe5c5d65f06	2415	TRUE	1	1	1	1	1	22	33	43	53.75	75	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Micropepsales;D_4__Micropepsaceae
0c7eed5c292f582dd090dc92af4b5c2c	2405	TRUE	1	1	1	1	1	19	38.25	42	51	71	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia;D_3__Gaiellales
5a1fd81ccdc96c8682313c4e79a38a97	2396	TRUE	1	1	1	1	1	18	36.25	42.5	48	57	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales
cf982f15528541ad7d0b7485d6e57cbd	2395	TRUE	17	31.75	39.5	45.75	51	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria
e44cf3cb7ba8dbd209635e91dc559ec3	2390	TRUE	1	1	1	1	1	32	33.5	35	40.25	59	D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Acidobacteriales;D_4__Acidobacteriaceae (Subgroup 1)
c5a2a3c42007dcc115e63314a18fe95a	2390	TRUE	19	27.25	28.5	35	38	1	1	1	1	1	D_0__Bacteria;D_1__Acidobacteria
ea1ca81ad7cec42500659ed2281f39c5	2384	TRUE	1	1	1	1	1	14	45.75	55	79.25	95	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Xanthomonadales;D_4__Rhodanobacteraceae;D_5__Rhodanobacter

*Reject null hypothesis: TRUE, indicates statistical significance.
**W statistic indicates the number of other items from which a single item is found to be significantly different.

APPENDIX M: α-Diversity estimators of seed-associated microbial communities in *Lens culinaris* lines.

Table M.1 α-Diversity estimators of *Lens culinaris* seed bacterial microbiomes from three generations.

Chao 1*			**Inverse Simpson's***		
Generation			**Generation**		
G0	G1	G2	G0	G1	G2
545.9**A**	40.8**B**	41.1**B**	19.10**A**	3.34**B**	4.05**B**

*Tukey post-hoc test ($p < 0.05$). Uppercase letters represent differences among generations.

Table M.2 α-Diversity estimators of *Lens culinaris* seed bacterial microbiomes from three generations harvested from two soils.

	Saskatoon						Scott					
	Chao 1*			Inverse Simpson's*			Chao 1*			Inverse Simpson's*		
	Generation			Generation			Generation			Generation		
Line	G0	G1	G2	G0	G1	G2	G0	G1	G2	G0	G1	G2
CDC KR-1	850**aA**	22.5 **bB**	30.3**B**	53.2**aA**	2.8 **abB**	2.5**B**	850**aA**	26.5 **bB**	19.5 **bB**	53.2**aA**	1.9 **cB**	2.3 **bB**
CDC Asterix	214**cA**	43 **abB**	20.3 **B**	3.8**c**	2.1**b**	1.5	214**cA**	15 **bB**	27.8 **bB**	3.8**c**	2.1 **bc**	2.2 **b**
CDC Marble	286 **bcA**	28.5 **bB**	19 **B**	6.8 **bc**	2.8 **ab**	2.1	286 **bcA**	75.2**aB**	42.8 **bB**	6.8 **bc**	4.3 **ab**	4.9 **b**
CDC QG-3	448.3 **bA**	45.8 **abB**	49.9 **B**	9.6 **bcA**	4.1 **abB**	2.8**B**	448.3 **bA**	53 **abB**	30.9 **bB**	9.6 **bcA**	5.2 **aB**	2.1 **bC**
Schwarze L.	404 **bcA**	52**abB**	35 **B**	12.1 **bcA**	3.8 **abB**	3.4 **B**	404 **bcA**	42.5 **abB**	34.2 **bB**	12.1 **bcA**	3.4**abcB**	3.4 **bB**
LR-30-32	816.2 **aA**	79**aB**	40 **B**	22**bcA**	5.7**aAB**	2.6**B**	816.2 **aA**	30.5**bB**	32.8 **bB**	22**bcA**	2.7 **bcB**	3.5**bB**
LR-30-101	804 **aA**	18.5 **bB**	68.5 **B**	26.3**bA**	2.6 **abB**	8**B**	804 **aA**	39.3 **abB**	125.3**aB**	26.3**bA**	3.1 **abcB**	15.4**aA**

*Tukey post-hoc test ($p < 0.05$). Lowercase letters represent differences among lines within a generation. Uppercase letters represent differences among generations.

APPENDIX N: Differences in seed-associated bacterial communities between *Lens culinaris* lines harvested from Scott soil.

Table N.1 Tukey post-hoc test of *Lens culinaris* line differences in seed-associated bacterial communities using the PCoA axes.

	Generation1*		Generation2*	
Line	**PCO1**	**PCO2**	**PCO1**	**PCO2**
CDC KR-1	-0.23**c**	-0.01**a**	-0.10**bc**	0.09 **a**
CDC Asterix	-0.07 **abc**	0.10**a**	-0.18**c**	0.02 **a**
CDC Marble	0.14**a**	-0.03**a**	-0.12**b**	-0.04 **a**
CDC QG-3	0.04 **abc**	-0.01**a**	0.07 **abc**	0.09 **a**
Schwarze L.	-0.05 **abc**	-0.0004**a**	0.13**abc**	0.06 **a**
LR-30-32	-0.18 **bc**	0.003**a**	0.19 **ab**	0.07 **a**
LR-30-101	0.10 **ab**	0.04**a**	0.26**a**	-0.4**b**

*Tukey post-hoc test ($p < 0.05$). Lowercase letters represent differences among lines within a generation.

APPENDIX O : Taxonomic assignment of the bacterial ASVs shared among all three generations of *Lens culinaris*.

Table O.1 Taxonomic assignment of the bacterial ASVs shared among all three generations of *Lens culinaris*.

Line	Saskatoon	Scott
CDC KR-1	**1. Acetobacteraceae** **2. Actinomycetales** **3. Xanthobacteraceae** **4. Sphingomonadaceae** **5. Bacillaceae (*Bacillus* sp.)** 6. Beijerinckiaceae (*Methylobacterium* sp.) 7. Beijerinckiaceae (*Methylobacterium* sp.) 8. Geodermatophilaceae	**1. Acetobacteraceae** **2. Actinomycetales** **3. Xanthobacteraceae** **4. Sphingomonadaceae** **5. Bacillaceae (*Bacillus* sp.)** 6. Chloroflexi 7. Brevibacteriaceae (*Brevibacterium* sp.)
CDC Asterix	**1. Actinomycetales** 2. Staphylococcaceae (*Staphylococcus* sp.) 3. Bacillaceae (*Bacillus* sp.) 4. Micrococcaceae	**1. Actinomycetales** 2. Sphingomonadaceae 3. Geodermatophilaceae
CDC Marble	**1. Actinomycetales** **2. Beijerinckiaceae (*Methylobacterium* sp.)** 3. Sphingomonadaceae 4. Enterobacteriaceae (*Erwinia* sp.) 5. Beijerinckiaceae (*Methylobacterium* sp.) 6. Gaiellaceae (*Gaiella* sp.)	**1. Actinomycetales** **2. Beijerinckiaceae (*Methylobacterium* sp.)** 3. Bacillaceae (*Bacillus* sp.) 4. Streptococcaceae (*Streptococcus* sp.) 5. Gaiellaceae (*Gaiella* sp.)
CDC QG-3	**1. Acetobacteraceae** **2. Actinomycetales** **3. Sphingomonadaceae** **4. Bacillaceae (*Bacillus* sp.)** **5. Micrococcaceae** **6. Gaiellales** 7. Chloroflexi 8. Solirubrobacterales 9. Nocardioidaceae (*Aeromicrobium* sp.) 10. Pseudonocardiaceae (*Pseudonocardia* sp.) 11. Beijerinckiaceae (*Methylobacterium* sp.) 12. Frankiales 13. Nocardioidaceae (*Nocardioides* sp.) 14. Gaiellaceae (*Gaiella* sp.) 15. Chitinophagaceae	**1. Acetobacteraceae** **2. Actinomycetales** **3. Sphingomonadaceae** **4. Bacillaceae (*Bacillus* sp.)** **5. Micrococcaceae** **6. Gaiellales** 7. Xanthobacteraceae 8. Sphingomonadaceae 9. Gemmatimonadaceae 10. Gaiellales
Schwarze L.	**1. Acetobacteraceae** **2. Actinomycetales** **3. Bacillaceae (*Bacillus* sp.)** 4. Beijerinckiaceae (*Methylobacterium* sp.) 5. Propionibacteriaceae (*Microlunatus* sp.) 6. Chloroflexi 7. Microbacteriaceae 8. Gaiellales 9. Bacillaceae (*Bacillus* sp.) 10. Gaiellales 11. Intrasporangiaceae	**1. Acetobacteraceae** **2. Actinomycetales** **3. Bacillaceae (*Bacillus* sp.)** 4. Sphingomonadaceae 5. Micrococcaceae
LR-30-32	**1. Acetobacteraceae** **2. Actinomycetales** **3. Sphingomonadaceae** **4. Streptococcaceae (*Streptococcus* sp.)** 5. Xanthobacteraceae 6. Rhodobacteraceae (*Paracoccus* sp.) 7. Staphylococcaceae (*Staphylococcus* sp.)	**1. Acetobacteraceae** **2. Actinomycetales** **3. Sphingomonadaceae** **4. Streptococcaceae (*Streptococcus* sp.)** 5. Sphingomonadaceae 6. Bacillaceae (*Bacillus* sp.) 7. Pseudomonadaceae (*Pseudomonas* sp.)

Line	Saskatoon	Scott
	8. Corynebacteriaceae 9. Gaiellales 10. Rubrobacteriaceae (*Rubrobacter* sp.) 11. Solirubrobacterales 12. Micrococcaceae 13. Blastocatellaceae 14. Acidobacteria 15. Actinobacteria 16. Caulobacteraceae (*Phenylobacterium* sp.) 17. Nocardioidaceae 18. Pseudonocardiaceae (*Pseudonocardia* sp.) 19. Acidobacteria	8. Spirosomaceae (*Dyadobacter* sp.) 9. Rhodobacteraceae (*Rubellimicrobium* sp.) 10. Gemmatimonadaceae 11. Staphylococcaceae (*Staphylococcus* sp.)
LR-30-101	**1. Burkholderiaceae (*Tepidimonas* sp.)** 2. Actinomycetales 3. Enterobacteriaceae 4. Micrococcaceae	**1. Burkholderiaceae (*Tepidimonas* sp.)** 2. Bacillaceae (*Bacillus* sp.) 3. Bacillaceae (*Bacillus* sp.) 4. Xanthobacteraceae 5. Staphylococcaceae (*Staphylococcus* sp.) 6. Sphingomonadaceae 7. Bacillaceae (*Bacillus* sp.) 8. Streptococcaceae (*Streptococcus* sp.) 9. Streptococcaceae (*Lactococcus* sp.) 10. Pseudomonadaceae (*Pseudomonas* sp.) 11. Pseudomonadaceae (*Pseudomonas* sp.) 12. Micrococcaceae 13. Caulobacteraceae 14. Nocardioidaceae (*Nocardioides* sp.) 15. Azospirillaceae (*Skermanella* sp.) 16. Propionibacteriaceae (*Microlunatus* sp.) 17. Chloroflexi 18. Corynebacteriaceae (*Corynebacterium* sp.) 19. Beijerinckiaceae (*Methylobacterium* sp.) 20. Bacillaceae (*Bacillus* sp.) 21. Chloroflexi 22. Enterobacteriaceae

Taxa in bold denote exact ASVs present in samples harvested from both soils. Taxonomy was assigned using the SILVA database v.132.